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**EARLY ENVIRONMENTS AND NEUROBEHAVIOURAL
PROGRAMMING: THERAPEUTIC ACTIONS OF
ANTIDEPRESSANTS**

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PhD

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**EARLY ENVIRONMENTS AND NEUROBEHAVIOURAL PROGRAMMING:
THERAPEUTIC ACTIONS OF ANTIDEPRESSANTS**

Neurobehavioural programming during development

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ABSTRACT

EARLY ENVIRONMENTS AND NEUROBEHAVIOURAL PROGRAMMING: THERAPEUTIC ACTIONS OF ANTIDEPRESSANTS

Ali Alrumaih

Keywords: Stress, Maternal Behaviour, Offspring, Hippocampus, Depression, Antidepressant.

Following decades of research on stress and its impact on behaviour, it is now widely accepted that selective psycho-pathologies, in particular clinical depression are more prevalent in humans with prior history of life-stress events. Interest in stress has led to questions about how it might affect the physiology and behaviour of animals exposed indirectly during gestational development. Not unexpectedly gestational stress has been shown to affect the offspring in several ways: endocrine responses to stress are elevated, fear, arousal and affective disturbances are all subject to vary if the pregnant animal is subjected to periods of aversive stimulation.

Beginning in 1997, Michael Meaney of McGill University produced a series of publications suggesting that peri-natal events influence offspring and infant development, not via physical discomfort or physiological disturbance, but does so through modifications of maternal behaviour. Highly nurturant mothers (those who engage in active arched-back nursing (ABN), and spend more time licking and grooming (L/G) their pups), programme their offspring with improved cognitive abilities, decreased anxiety and fear, and reduced HPA axis hormone secretion. Low-nurturant mothers, who engage in less ABN and less L/G, tend

to programme the opposite responses in their offspring. Our initial foray into this field was to investigate if gestational stress might also produce responses in the offspring via changes in maternal behaviour, and indeed ABN and L/G were reduced in dams which were subjected to gestational stress.

We queried why stressed Dams would be less maternal towards their infants, and tested gestationally-stressed Dams in the Porsolt test for depressive-like behaviour. Our results suggested that these stressed Dams were actually depressed and this resulted in less maternal behaviour. Human mothers with depression are also less maternal and have been shown to divest themselves of infant care much like our prenatally-stressed Dams. On this basis we have proposed that gestational stress induced decrements in maternal behaviour represent a novel rat model for postnatal depression with face and construct validities.

In the present work we have attempted to replicate the findings of Smythe's group (Smith *et al.*, 2004), and have investigated the potential for antidepressants to alter the influence of gestational stress on maternal behaviours and depressive-like response, and whether or not the offspring are modified by maternal treatment with ant-depressants.

Approximately 140 time-mated, lister hooded rats were generated in house, and subjected to gestational stress on days 10-20 (1hr restraint/day) or remained undisturbed in their home cages. Following birth, cohorts of control and stressed Dams were administered vehicle or an antidepressant (imipramine 15mg/kg; or sertraline 10mg/kg) once daily until postnatal day 10.

We assessed maternal Porsolt activity, nurturance (ABN, L/G, nest building) and anxiety-like behaviour in the elevated plus maze (EPM). Representative

offspring of each Dam's treatment conditions were maintained post weaning and assessed in the Porsolt and EPM to determine if any changes in maternal behaviour elicited by the antidepressants altered their behavioural programming.

Our findings confirm that Dams show depressive-like symptoms following gestational stress, and that administration of antidepressants to the Dams reduces depressive-like behaviour and increased maternal care.

We propose that rat gestational stress is a putative model for human postnatal depression. Prenatal stress effects on maternal behaviour in the rat Dam represent a novel, and innovative model for human postnatal depression.

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Table of Contents

CHAPTER ONE: INTRODUCTION	1
1. INTRODUCTION.....	2
1.1 STRESS OVERVIEW	2
1.1.1 ANATOMY AND PHYSIOLOGY OF THE STRESS SYSTEM.....	5
1.1.2 SUBTYPES OF STRESS.....	6
1.2 THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS.....	11
1.2.1 THE ROLE OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS	11
1.2.2 PATHOPHYSIOLOGICAL CHANGES IN HYPOTHALAMO-ADRENAL-AXIS AXIS AND ITS RELATED CONSEQUENCES	22
1.2.3 STRESS AND NEURODEGENERATIVE DISORDERS	25
1.2.4 STRESS AND EARLY LIFE EXPERIENCE	26
1.2.5 THE DEVELOPMENTAL PROFILE OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS.....	28
1.3 PRENATAL MANIPULATIONS: NEUROCHEMICAL AND BEHAVIOURAL RESPONSES OF OFFSPRING AND MOTHERS	32
1.3.1 IMPACT OF PRENATAL STRESS ON DEVELOPMENT	32
1.3.2 PRENATAL STRESS AND HYPOTHALAMO-PITUITARY-ADRENAL AXIS CHANGES.....	34
1.3.3 PRENATAL STRESS AND CENTRAL AMINERGIC SYSTEMS	37
1.3.4 PRENATAL STRESS AND BEHAVIOUR	39
1.3.5 PRENATAL STRESS AND SEXUAL BEHAVIOUR.....	41
1.4 POSTNATAL MANIPULATIONS: NEUROCHEMICAL AND BEHAVIOURAL RESPONSES IN MOTHERS AND OFFSPRING	43
1.4.1 POSTNATAL ENVIRONMENT AND THE HYPATHALAMO-PITUITARY-ADRENAL AXIS	43
1.4.2 MATERNAL CARE AND OFFSPRING DEVELOPMENT	45
1.5 DEPRESSION.....	48
1.5.1 OVERVIEW.....	48
1.5.2 DEPRESSION – FACTS AND FIGURES.....	49

1.5.3	IMPACT OF DEPRESSION	50
1.5.4	CAUSES OF DEPRESSION.....	52
1.5.5	CLASSIFICATION OF DEPRESSION	54
1.5.6	RISK FACTORS FOR DEPRESSION	55
1.5.7	STRESS AND DEPRESSION.....	56
1.5.8	PATHOPHYSIOLOGY AND PHARMACOLOGY OF DEPRESSION	58
1.5.9	TREATMENT OF DEPRESSION	61
1.6	ANIMAL MODELS	68
1.6.1	GENERAL CONSIDERATIONS IN MODELLING DEPRESSION.....	68
1.6.2	ANIMAL MODELS OF DEPRESSION	70
1.6.3	VALIDITY OF ANIMAL MODELS OVERVIEW	72
1.6.4	TYPES OF ANIMAL MODELS OF DEPRESSION	75
1.6.5	ANIMAL MODELS OF ANXIETY.....	85
1.7	PROGRAMMING.....	86
1.7.1	OVERVIEW.....	86
1.7.2	DEFINITIONS OF PROGRAMMING	90
1.7.3	MECHANISMS OF PROGRAMMING	90
1.8	MATERNAL BEHAVIOUR.....	95
1.8.1	DESCRIPTION OF RAT MATERNAL BEHAVIOUR	95
1.9	AIMS AND OBJECTIVES	98
1.9.1	BACKGROUND	98
1.9.2	AIMS	98
1.9.3	OBJECTIVES.....	99
CHAPTER TWO: MATERIALS AND METHODS		101
2.	MATERIALS AND METHODS	102
2.1	ETHICAL APPROVAL.....	102
2.2	HOUSING AND HUSBANDRY.....	102

2.3	MATING.....	103
2.4	SMEARING.....	103
2.5	HOUSING OF PREGNANT RATS.....	104
2.6	GESTATIONAL STRESS	105
2.6.1	<i>PROCEDURE</i>	105
2.7	PRENATAL PORSOLT.....	109
2.7.1	<i>PROCEDURE</i>	109
2.8	2.8 EXPERIMENTAL GROUPS.....	111
2.8.1	<i>MOTHERS</i>	112
2.8.2	<i>WEANING</i>	114
2.8.3	<i>OFFSPRING</i>	115
2.9	POSTNATAL PORSOLT MOTHERS	116
2.9.1	<i>PROCEDURE</i>	116
2.10	OFFSPRING PORSOLT.....	117
2.10.1	<i>PROCEDURE</i>	117
2.11	ELEVATED PLUS MAZE (EPM).....	118
2.11.1	<i>APPARATUS</i>	118
2.11.2	<i>PROCEDURE</i>	118
2.12	MATERNAL BEHAVIOURS ASSESSMENT	120
2.13	ASSESSMENT OF MATERNAL NESTING	124
2.14	STATISTICAL ANALYSIS.....	125
	CHAPTER THREE: RESULTS	127
3.	RESULTS	128
3.1	EFFECTS ON WEIGHTS	128
3.1.1	<i>EFFECT OF PRENATAL STRESS ON RAT GESTATIONAL WEIGHTS</i>	128
3.1.2	<i>POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF SERTRALINE</i>	129
3.1.3	<i>3.1.3 POSTNATAL DAM WEIGHTS AND THE EFFECTS OF IMIPRAMINE</i>	131

3.1.4	<i>OFFSPRING WEIGHT AND THE EFFECTS OF SERTRALINE</i>	133
3.1.5	<i>OFFSPRING WEIGHTS AND THE EFFECTS OF IMIPRAMINE</i>	135
3.2	<i>PRENATAL PORSOLT TEST IN PREGNANT DAMS</i>	137
3.2	<i>POSTNATAL PORSOLT DATA FOR DAMS AND THE EFFECTS OF IMIPRAMINE</i>	139
3.3	<i>OFFSPRING PORSOLT AND THE EFFECTS OF IMIPRAMINE</i>	141
3.3.1	<i>OFFSPRING PORSOLT AS STRESSED AND CONTROL</i>	147
3.4	<i>EFFECTS OF GESTATIONAL STRESS ON POSTNATAL ANXIETY IN DAMS</i>	149
3.4.1	<i>EFFECTS OF IMIPRAMINE</i>	149
3.4.2	<i>OFFSPRING ELEVATED PLUS MAZE AND THE EFFECTS OF SERTRALINE</i>	161
3.5	<i>MATERNAL BEHAVIOUR: EFFECTS OF GESTATIONAL STRESS AND TREATMENT WITH IMIPRAMINE</i>	170
3.5.1	<i>ARCHED BACK NURSING (ABN)</i>	170
3.5.2	<i>SUPINE NURSING</i>	172
3.5.3	<i>LICKING/GROOMING PUPS (LG)</i>	174
3.5.4	<i>PUP RETRIEVAL</i>	176
3.5.5	<i>TIME SPENT IN THE NEST</i>	178
3.5.6	<i>TIME SPENT OUT OF THE NEST</i>	180
3.5.7	<i>TIME SPENT IN NEST BUILDING</i>	182
3.5.8	<i>DAMS SELF GROOMING INSIDE THE NEST</i>	184
3.5.9	<i>DAMS SELF GROOMING OUT OF THE NEST</i>	186
CHAPTER FOUR: DISCUSSION		189
4.	DISCUSSION	190
4.1	<i>EFFECTS ON WEIGHTS</i>	190
4.1.1	<i>EFFECT OF PRENATAL STRESS ON RAT GESTATIONAL WEIGHTS</i>	190
4.1.2	<i>POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF SERTRALINE</i>	191
4.1.3	<i>POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF IMIPRAMINE</i>	192
4.1.4	<i>OFFSPRING WEIGHT AND THE EFFECTS OF SERTRALINE</i>	193
4.1.5	<i>OFFSPRING WEIGHT AND THE EFFECTS OF IMIPRAMINE</i>	196

4.2	PRENATAL PORSOLT	199
4.2.1	<i>PRENATAL PORSOLT TEST IN PREGNANT DAMS</i>	199
4.3	POSTNATAL PORSOLT (IMIPRAMINE).....	202
4.4	OFFSPRING PORSOLT (IMIPRAMINE)	209
4.5	EFFECTS OF GESTATIONAL STRESS ON POSTNATAL ANXIETY IN DAMS	213
4.5.1	<i>EFFECTS OF IMIPRAMINE</i>	213
4.6	OFFSPRING ELEVATED PLUS MAZE AND THE EFFECTS OF SERTRALINE	218
4.7	MATERNAL BEHAVIOUR: EFFECTS OF GESTATIONAL STRESS AND TREATMENT WITH IMIPRAMINE.....	220
CHAPTER FIVE: GENERAL DISCUSSION.....		227
5.	GENERAL DISCUSSION.....	228
REFERENCES		243
APPENDIX		305

LIST OF FIGURES

Figure 1-1: Schematic showing the main parts of the stress system. Hypothalamic-Pituitary-Adrenal (HPA) axis and the sympathetic adrenomedullary systems (SAM).....	6
Figure 1-2: Hypothalamic-Pituitary-Adrenal (HPA) Axis.	18
Figure 1-3: Chemical structure of the tricyclic antidepressant (imipramine).....	63
Figure 2-1: Diagram illustrating the sequences and procedures involved in the preparation of pregnant Dams, and the various manipulations which they were subjected to restrain stress.	107
Figure 2 2: Pictures showing open restrainers and the restraining area after adjusting the rubber blocks.....	108
Figure 2-3: Picture showing pregnant rats undergoing restraint.....	108
Figure 2-4: Picture showing water chamber used in Porsolt test.....	111
Figure 2-5: Picture showing experimental animal under the heat lamp. Pregnant dam or offspring were kept for ten minutes to be dried completely.....	111
Figure 2-6: Picture showing control mother with brown and yellow sizzle bedding....	113
Figure 2-7: Picture showing stressed mother with brown and orange sizzle bedding.	113
Figure 2-8: Schematic diagram of the Porsolt video recording system.....	117
Figure 2-9: Picture showing the elevated plus maze with dimensions for open arms, closed arms and the height used for testing rat mothers and their offspring.	

Underneath the maze we positioned soft padding material to eliminate harm to any rat that fell off the maze.....	119
Figure 2-10: Picture showing Arched back nursing (ABN).....	121
Figure 2-11: Picture showing an example of supine nursing.....	121
Figure 2-12: Picture showing mother absent from her nest.....	122
Figure 2-13: Picture showing the mother retrieving a pup.....	122
Figure 2-14: Picture showing mother were building her nest.....	123
Figure 2-15: Picture showing the quadrant divisions.1 as top left, 2 as top right, 3 as bottom right, 4 as bottom left, and 5 as central of the cage.....	124
Figure 2-16: Diagram illustrating the Cage specifications.it shows 1 as top left, 2 as top right, 3 as bottom right, 4 as bottom left, and 5 as central of the cage ,numbers 1 and 2 assigned as the distance from water and food hopper wither closed or far.....	125
Figure 3-1: Mean Cumulative weight gain (g) during gestational days 11-19, for control and gestationally-stressed Dams.	128
Figure 3-2: Mean postnatal cumulative weight gain (g) of Dam's days, 1-10 after giving birth.	130
Figure 3-3: Mean postnatal cumulative weight gain (g) of Dams from days 1-10.	132
Figure 3-4: Mean pups cumulative weight gain (g) from days 1-10.	134
Figure 3-5: Mean pups cumulative weight gain (g) from days 1-10.	136
Figure 3-6: Shows the Prenatal Porsolt data from control and stressed Dams at Days 17 and 18 of gestation (Exposure Day and Experimental Day).	138

Figure 3-7: Shows data for the control and stressed Dams following birth.	140
Figure 3-8: Immobility times for the Porsolt task recorded from the offspring (male and female) of gestationally stressed or control rat Dams following postnatal drug or vehicle treatment of the Dams immediately after birth.....	143
Figure 3-9: Offspring immobility times (from gestationally stressed or control Dams postnatally treated with Imipramine 15mg/kg or Vehicle (saline) after giving birth.	146
Figure 3-10: Immobility times for the Porsolt task recorded from the offspring (Males and female) of gestationally stressed or Control rat Dams on the Exposure and Experimental Days when offspring were 40-50 days old.	148
3-11: Effects of gestational stress and postnatal drug treatment on initial times to enter the open arms on days 4 and 10 of the elevated plus maze test.	150
Figure 3-12: Effects of gestational stress and postnatal drug treatment on initial times to enter the closed arms on days 4 and 10 of the elevated plus maze test.	152
Figure 3-13: Effects of gestational stress and postnatal drug treatment on time spent grooming in the open arms for Dams tested in the elevated plus maze on postnatal days 4 and 10.	154
Figure 3-14: Effects of gestational stress and postnatal drug treatment on time spent grooming in the closed arms for Dams tested in the elevated plus maze on postnatal days 4 and 10.	156
Figure 3-15: Effects of gestational stress and postnatal drug treatment on total time in the open arms the elevated plus maze for Dams tested on postnatal days 4 and 10.	158

Figure 3-16: Effects of gestational stress and postnatal drug treatment on total time in the closed arms the elevated plus maze for Dams tested on postnatal days 4 and 10.	160
Figure 3-17: Shows offspring time to enter the open and closed arms.	163
3.4.2.3 Figure 3-18: Shows offspring time spent grooming in the open and closed arms. These were born of Dams which had been left undisturbed during gestation (Control), or who had been subjected to gestational stress. At birth Dams were treated with once daily injections of saline or sertraline (10mg/kg) up to and including postnatal day 10.al Times spent in open and closed arms	166
3-19: Shows offspring amount of total time spent in the open and closed arms.	169
Figure 3-20: Arched-Back nursing times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.	171
Figure 3-21: Supine nursing times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.....	173
Figure 3-22: Licking/Grooming times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.	175
Figure 3-23: Time spent by Dams engaged in actual pup retrieval during the 30 minute observation period.	177
Figure 3-24: Time spent in Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.....	179

Figure 3-25: Time spent out of Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.....	181
Figure 3-26: Nest Building times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.....	183
Figure 3-27: Self Grooming times in Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.	185
Figure 3-28: Self Grooming times out of Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.....	187
Figure 3-29: Schematic Summary of Results.	188

LIST OF TABLES

Table 1.1: Properties of Mineralocorticoid and Glucocorticoid receptors in the brain...	16
Table1- 2: Signs and symptoms of depression.	49
Table 1.3: Reasons for using animals in the study neurobehavioural function.	70

LIST OF ABBREVIATIONS

5HT.....	5-hydroxytryptamine
5HIAA.....	5-hydroxyindole acetic acid
ABN.....	Arched back nursing
ACTH.....	Adrenocorticotrophic Hormone
ANOVA.....	Analysis of variance
AOB.....	Area of bedding
AVP.....	Arginine Vasopressin
BNST.....	Bed nucleus of the Stria Terminalis
BSU.....	Biological service unit
CBG.....	Corticotropin binding globulin
CeA.....	Central nucleus of the amygdala
CNS.....	Central Nervous System
CORT.....	Corticosterone/Cortisol
CRF.....	Corticotrophin Releasing Factor
CSF.....	Cerebrospinal Fluid
DA.....	Dopamine
DRN.....	Dorsal Raphé Nucleus
EPM.....	Elevated Plus Maze
FH.....	Food Hopper
FST.....	Forced Swim Test
GAD.....	Generalized Anxiety Disorder
GC.....	Glucocorticoid
GR.....	Glucocorticoid Receptor
HPA.....	Hypothalamo-Pituitary-Adrenal Axis
LC.....	Locus Coeruleus

LG.....	Licking/Grooming
LH.....	Learned helplessness
MC.....	Mineralocorticoid
MAOI.....	Monoamine oxidase inhibitor
MPO.....	Medial Preoptic Area
mRNA.....	messenger Ribonucleic Acid
MR.....	Mineralocorticoid Receptor
NA.....	Noradrenaline
NB.....	Nest Building
OCD.....	Obsessive–Compulsive Disorder
OXY.....	Oxytocin
P:NS.....	Not Significant
POMC.....	Pro-opiomelanocortin
PPD.....	Postpartum Depression
PS.....	Prenatal Stress
PVN.....	Paraventricular Nucleus
SAM.....	Sympathetic Adrenomedullary
SSRI.....	Selective Serotonin Reuptake Inhibitor
SON.....	Shape of the nest
TCA.....	Tricyclic Antidepressant
USV.....	Ultrasonic Vocalisation
VTA.....	Ventral Tegmental Area

CHAPTER ONE: INTRODUCTION

1. INTRODUCTION

1.1 STRESS OVERVIEW

Every day human beings experience various challenges, consciously or unconsciously that are often referred to as 'stress'. These challenges may affect us physiologically or psychologically; or as a combination of both. Physical stressors include restraint, heat, cold, noise, electric foot shock, and swimming. Identifying and defining stress scientifically is a challenging task. Many definitions and ideas characterising stress have been accepted but the most notable definition originates with Hans Selye, often considered the father of modern stress research. Selye (1956) defined stress in physico-mechanistic terms as "the rate of wear and tear in the body" such as the tiredness experienced after a day of strenuous work, feeling anxious or becoming ill. These all create sensations of stress. Until recently the concept of stress had been left unaltered due to the lack of identifiable markers to measure it as well as the lack of metrics to assess its impact on humans. Realising that, stress is not only an adaptive reaction but also impacts individuals differently.

Chrousos et al. (1992) reported that Thomas Sydenham (1665) suggested that an individual's adaptive response to such forces could itself be capable of producing pathological changes (Chrousos, 1992).

Claude Bernard (1813–78) pioneered the doctrine of stability of *milieu interieur* otherwise known as homeostasis (Selye, 1973b). In the middle of the Twentieth Century, Hans Selye and Walter Cannon both proposed concepts of stress and discussed the relationship between diseases and stress reactivity with respect

to homeostasis (Rosmond, 2005). Walter Cannon (1871–1945), in his 1929 report promoted the concept of physiologic organisation and regulations involved in stress and homeostasis. Homeostasis can be defined as the maintenance of steady state conditions in biological systems or as the regulation of optimal conditions the body needs to maintain consistent functioning in biological systems e.g. nervous system, endocrine system, muscular system, circulatory system, reproductive system, digestive system lymphatic system, respiratory system and skeletal system by means of automatic mechanisms (Chrousos, 2009). It has been proposed that feedback systems act to buffer influences (stressors) that tend towards disequilibrium. In order to maintain homeostasis, the body has internal mechanisms to maintain constant functioning and equilibrium of these systems at a stable state. Once the individuals are exposed to different challenges, the body reacts to each of these challenges by adjusting its different physiological systems in order to recover from these challenges. For example, the body releases more waste fluid by excessive urination and sweating when an organism is “under stress”. To counteract this effect, increased water intake; fluid regulation is required to maintain body equilibrium (Cannon, 1939; Chrousos, 2009).

Cannon in his pioneering work explored the sympathetic-parasympathetic nervous system response to an immediate stressor. Individuals responded to danger through Sympathetic Nervous System (SNS) arousal, causing significantly increasing respiration rate and heart rate. The body's compensatory response involves either reducing SNS activity or increasing parasympathetic nervous system as counter-activity. Hans Selye published his

synthetic theory of the General Adaptation Syndrome (GAS) and diseases of adaptation (Selye, 1946). He defined GAS as the "physiological mechanism which raises the resistance to damage" and proposed a three-stage reaction to stress; The first stage is an alarm reaction which is then followed by a resistance reaction and concludes with an exhaustion stage. Importantly, Selye characterised the release of hormones (glucocorticoids) from the adrenal cortex and the role of medullary hormones (adrenaline, noradrenaline) in mediating these three stages of response (Selye, 1973a).

Selye (1956) further defined stress as responses to demands placed on the body. Although numerous other definitions defining stress also exist, perhaps the most notable is by Burchfield (1979). Burchfield specified that psychological factors as well as physical factors could play an important contributing factor to stress (Burchfield, 1979).

John Mason (Mason, 1968; Mason, 1975) pointed out that stress is triggered by activating the pituitary-adrenocortical system and the most potent stimuli for activating the pituitary-adrenocortical system are; (a) the physiological and psychological reactions to perceived internal states of the organism; (b) natural disasters, trauma and major life events, i.e. external factors; and (c) early life experiences such as poverty, poor education and poor social conditions through infancy and childhood (Mason, 1975; Mason, 1968).

Sterling and Eyer (1988) studied stress and introduced the concept of allostasis, a process by which the body responds to stressors thus allowing it to regain its basal activity (Sterling and Eyer, 1988). McEwen and Stellar (1993) broadened this concept further and promoted allostasis as a process that maintained

stability through change and by enabling adaptation (McEwen and Stellar, 1993; Flier *et al.*, 1998; McEwen, 1998; McEwen, 2000; Seeman *et al.*, 2001; McEwen, 2007).

1.1.1 ANATOMY AND PHYSIOLOGY OF THE STRESS SYSTEM

The central components of the stress system are located in the hypothalamus and the brain stem, the neural systemic sympathetic-adrenomedullary (SAM) and the endocrine hypothalamic-pituitary-adrenal (HPA) axis (Figure 1-1). These systems act to receive information from both internal and external origins and compute an appropriate response. Their central regulators and peripheral end products include catecholamines (adrenaline and noradrenaline), corticotropin releasing hormone (CRF) and glucocorticoids; these are key hormones in the maintenance and re-establishment of cardiovascular, metabolic, behavioural and immune homeostasis (Fink, 2000).

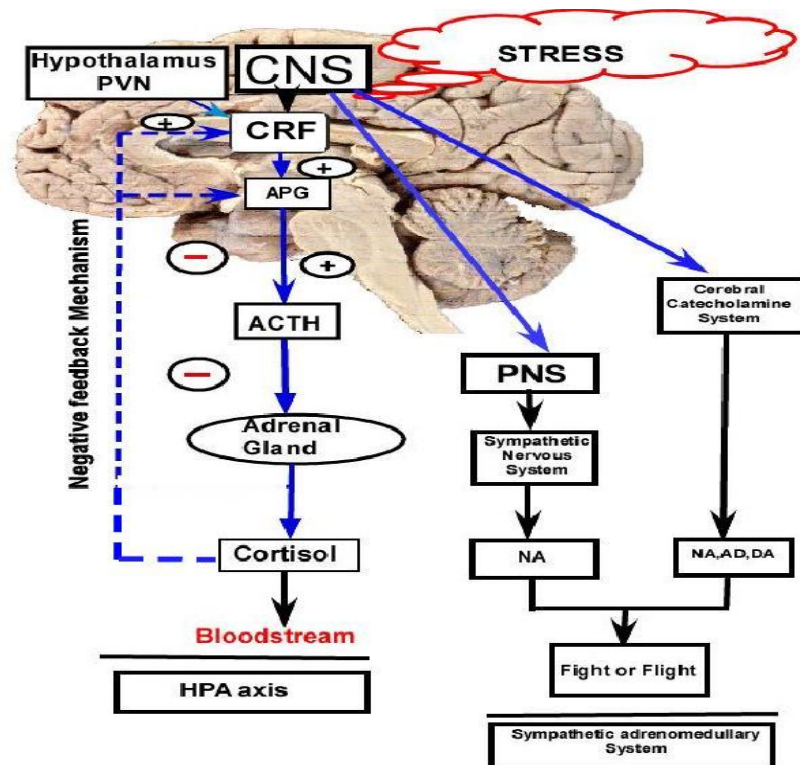


Figure 1-1: Schematic showing the main parts of the stress system. Hypothalamic-Pituitary-Adrenal (HPA) axis and the sympathetic adrenomedullary systems (SAM). PVN=paraventricular nucleus; CRF=corticotropin releasing factor; ACTH=adrenocorticotrophic hormone; APG=anterior pituitary gland; CNS=central nervous system; PNS=peripheral nervous system; NA=noradrenaline; AD=adrenaline; DA=dopamine.

1.1.2 SUBTYPES OF STRESS

The fact that there are different types of stress complicate the issue of stress management. Broadly, stress can be divided into two main categories: acute and chronic.

1.1.2.1 Acute

Acute stress is one of the most common forms of stress reactivity. It arises from a transient and brief exposure to an immediately demanding event. Responses to acute stress are normal and healthy, and generally assist the individual during the stressful event to mount and maintain a response. Examples might include meeting deadlines, missing a train or falling over.

Complexity emerges when considering the magnitude and consequences which arise from an acute stress episode. Many acute stressors are unexpected and undesired by the individual. The examples above illustrate unpredictable and negative acute stressors where controls over the stressful events are beyond the individual's powers of influence. These stressors may cause brief distress or fear and highlight the importance of a person's perceptions of such events. If we extend our examples we may consider how an individual perceives a visit to the dentist. While consciously acknowledging the importance of oral and dental health, routine procedure such as a cleaning and fillings may cause pain. This creates a sense of dread and temporary helplessness in the individual. Whilst the event itself lasts only briefly, the process of attending the clinic, the visual and auditory cues and the presence of other patients exhibiting visible worry can become associated with each and every subsequent visit to the dentist. This shows that the acute stressors and the individual's reaction to them are influenced by previously learned associations and anticipatory fears (Mack *et al.*, 1998; Lerner *et al.*, 2007). Thus, one's perception of a transient event

includes prior experience and memory and therefore the actual physical event and pain of dental procedures may provoke these feelings.

However, other events may also elicit acute stress responses but can be perceived positively and entirely under the control of the individual especially if the individual engages in the activity deliberately. For example, parachuting is a sporting/thrill-seeking behaviour. The individual makes a conscious decision to participate in this activity. It brings with it a heightened sense of excitement and anticipation; the exhilaration of free-falling and great relief/enjoyment upon a safe and successful landing. The event is planned and predicted with a diminished degree of unknowns involved. This form of acute stress is termed positive stress and can be a source of great joy, engagement with a shared group experience, and can positively broaden an individual's experiential repertoire (Nelson and Cooper, 2005; Little *et al.*, 2007).

Both negative and positive stressors produce many identical physiological reactions involving the sympathetic nervous system and HPA axis activity. Psychologically, both types of stressors may produce equivalent degrees of fear and apprehension. The crucial important distinction between them lies in the notion of how the individual perceives the stress and reacts to it upon re-exposure: if it is negative and unpleasant it will be avoided; if it is positive and reinforcing it will be welcomed or indeed even sought after (Nelson and Cooper, 2005; Little *et al.*, 2007).

Levels of control and predictability become markers for positive and negative stressors. Minimal identifiable harm to the individual follows any type of acute stress. This is not the case when the stress is chronic in nature, particularly for

negative stressors which may underpin deleterious consequences for individuals, as will be described below (Mack *et al.*, 1998; Nelson and Cooper, 2005; Little *et al.*, 2007).

Acute stress mechanism

When an acute stress occurs, impulses to the hypothalamus prompt the production of corticotropin-releasing factor (CRF) and vasopressin (AVP) which in turn activates the release of adrenocorticotrophic hormone (ACTH), and ACTH leads to the rapid mobilization of glucocorticoids (GCs). If the stress is protracted, the HPA axis generally adapts to it such that the ACTH and GCs return to baseline levels quickly. In rats, it has been shown that the response of GCs to stress in later life can be modified by neonatal handling, which appears to increase the efficiency of negative-feedback control of the stress response, protecting the animal from the potentially damaging effects of GCs sustained exposure (Fink, 2000). This appears to occur as a result of augmented GC receptor densities in the hippocampus which promote greater negative feedback control over the HPA axis (Meaney *et al.*, 1985).

1.1.2.2 Chronic stress

Chronic stress is generally viewed negatively. This form of stress occurs day after day, year after year and is thought to result in pathologies (Selye, 1946). Logically it is the stress of poverty, of dysfunctional families, of feeling trapped in abusive relationships or of struggling to find suitable and satisfying employment (Selye, 1956).

Chronic stress arises when a person fails to arrive at a solution for miserable situations (Selye, 1956). It is the stress of persisting demands and pressures for seemingly indefinite periods of time. The affected individual often gives up looking for solutions and places themselves at risk of various illnesses, diseases, or psychopathologies (Coyne and Downey, 1991; Patacchioli *et al.*, 2001).

Chronic stress mechanism

Most animal models of chronic stress are characterized by persistent neuroendocrine, immunological and behavioural changes. For instance, the entire HPA axis is activated, the thyroid and reproductive axes are suppressed, growth hormone is suppressed and prolactin release is stimulated (Ottenweller, 2000). Most studies report immune suppression after chronic stress but there can also be an enhanced immune function; for example, chronic stress will exacerbate pathologies in experimental allergic-encephalomyelitis, an autoimmune animal model for multiple sclerosis (Ottenweller, 2000). The behavioural changes that are reported include hyperarousal as indicated by increased startle responses, fear of novelty and decreased exploration in open field tasks (Ottenweller, 2000). In addition, chronic stress can inhibit spatial memory in the Morris Water Maze but can also enhance the acquisition of a classically conditioned eye-blink response (Ottenweller, 2000). Thus the effects on learning and memory may depend on the kind of stressor and the type of learning being tested.

Although studies conflict, with some showing activation and others attenuation of HPA axis function following chronic stress, most studies reveal abnormalities of cortisol secretory dynamics (Fink, 2000).

Evidence suggests that during chronic stress, the CRF: AVP ratio may increase. This could possibly be due to differential sensitivity of the secretagogues to negative-feedback regulation (Scott and Dinan, 1998; Scott and Dinan, 2002; Dinan *et al.*, 2004; Raadsheer *et al.*, 2008).

Physical or mental stress causes increased activity in the limbic system, especially in the region of the amygdala and hippocampus which in turn transmit signals to the hypothalamus. These signals cause greatly enhanced secretion of CRF (corticotropin releasing factor) into the hypophyseal portal system which in turn stimulates greater release of ACTH (adrenocorticotrophic hormone) (Swaab *et al.*, 1992; Meaney *et al.*, 1993; Plotsky, 2006).

Adrenocorticotrophic hormone promotes the rapid synthesis and secretion of adrenal cortisol/corticosterone. Impairment of the negative feedback loop at this stage could account for the inability of the glucocorticoid to regulate its own secretion during chronic stress (Chrousos *et al.*, 1998; McEwen, 1998; Charmandari *et al.*, 2005; Chrousos and Kino, 2007; Chrousos, 2009).

1.2 THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

1.2.1 THE ROLE OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

The hypothalamo-pituitary-adrenal (HPA) axis is widely recognized and accepted as a key physiological system through which an organism triggers a response to stress. Selye (1956) defined stress as responses to demands

placed on the body but it can also be defined as alterations in psychological homeostatic processes (Burchfield, 1979). This system has a basal level of activity which, like many hypothalamically-regulated systems, maintains a strict diurnal rhythm (Spangler, 1991).

While a notable rhythmical pattern is present in the newborn, the adult-like circadian rhythm of cortisol develops during the first six months and occurs in parallel with the 24 hour sleep-wake cycle (Spangler, 1991). There is a fluctuation of plasma cortisol rhythmic activity level corresponding with the expected rise in motor activity which reaches its peak in the morning and declines prior to sleep. Corticosterone (CORT) levels in nocturnal animals such as rodents exhibit a distinct circadian variation with peak values in the later portion of day followed by a nadir in the morning. This is believed to play an important role in their sleep-wake cycle. Enhanced corticosterone release by female rats compared to male rats under basal and stress conditions have been observed (Handa *et al.*, 1994; Bingaman *et al.*, 2008). It is also obvious that while the rhythm is under the influence of light and dark cycles, it is also affected by water and food intake (Krieger, 1974; Krieger and Hauser, 1978; Wilkinson *et al.*, 1979; Roland *et al.*, 2008). In rats, 20-fold differences in plasma levels of corticosterone concentration occur between the zenith and nadir over a 24-hour period. This is thought to arise from two factors: (a) a raised adrenal sensitivity to adrenocorticotrophic hormone (ACTH); and (b) by an augmentative effect of corticotropine releasing factor (CRF) upon ACTH plasma secretion (Dallman, 1993).

The HPA axis shows significant changes in both threshold and magnitude of activation in addition to altered activity corresponding to the circadian rhythm. This reflects the importance of the HPA axis responses in maintaining homeostasis especially with respect to its impact on metabolic and immunological activity (Brindley, 1981; Munck *et al.*, 1984). In evolutionary terms, it helps animals to enable and utilise physiological defence mechanisms to preserve life. CORT further stimulates the production of additional energy substrates through restricting insulin actions and enhancing glucagon production (Barseghian and Levine, 1980). Consequently, continuous exposure to stress induces catabolic effects upon the body. Such exposures can trigger specific alterations to the functioning capacities of the HPA axis. It has been reported that chronic activation of the HPA axis has been associated with hyperlipidaemia, muscle atrophy, steroid-induced diabetes, hypertension and central neurone loss (Meaney *et al.*, 1983; Munck *et al.*, 1984; Brindley and Rolland, 1989).

1.2.1.1 The physiology of the HPA axis

The sequential nature of HPA axis activation has been well documented in the adult rat. Acute stress stimulates the paraventricular nucleus (PVN) of the hypothalamus to increase the secretion of corticotrophin-releasing-factors (CRF) and arginine-vasopressin (AVP) into the capillary bed of the median eminence (Antoni, 1986; de Goeij *et al.*, 1992; Whitnall, 1993; De Goeij *et al.*, 2008). These secretagogues act within the anterior pituitary where they stimulate the cleavage of pro-opiomelanocortin (POMC) into

adrenocorticotrophic hormone (ACTH), β -endorphin (β -END), α -melanocyte stimulating hormone (α -MSH) and various enkephalin-related peptides (for review, see Meaney *et al.* 1993). Following release into the circulatory system, ACTH acts on the adrenal gland to upregulate the rapid synthesis and secretion of CORT (Gunnar and Quevedo, 2007). The precise roles of the other POMC fragments have yet to be elucidated. CRF neurones are widely distributed in the Central Nervous System (CNS) including regions other than the hypothalamus, such as the prefrontal and cingulate cortices, the central nucleus of the amygdala (CeA) and the hippocampus (Owens and Nemeroff, 1991; Sawchenko *et al.*, 1993; Champagne *et al.*, 1998; Silverman *et al.*, 2008). Interestingly, CRF perikarya are localised in brainstem regions including the locus coeruleus (LC), the dorsal raphe nucleus (DRN) and midbrain areas such as the ventral tegmental area (VTA). This suggests a possible role for CRF in the modulation of noradrenaline (NA), serotonin (5HT) and dopamine (DA) projection systems, respectively (De Souza *et al.*, 1985; Valentino *et al.*, 1992; Champagne *et al.*, 1998; Valentino and Wehby, 2008). Evidence has led many to suggest that CRF influences not only endocrine, but also autonomic and immunological responses (Butler *et al.*, 1990; Owens and Nemeroff, 1991; Koob *et al.*, 1994; De Souza, 1995). It is therefore unsurprising that Cerebrospinal Fluid (CSF) levels of CRF have been shown to be asynchronous with HPA activity (Garrick *et al.*, 1987), possibly a reflection of the difference between endocrine and non-endocrine functions of CRF systems within the CNS (Oldfield *et al.*, 2008).

Other than the varied putative roles for CRF in neurochemical activation, it is established that the CRF neurones of the parvocellular region of the PVN represent the principle hypothalamic component of the HPA axis, i.e. the higher centre of control mediating the physiological stress response (Dallman *et al.*, 1994). In order to co-ordinate HPA axis activity with the bodies demands, the PVN receives numerous inputs, particularly from the dorsomedial nucleus of the hypothalamus and the bed nucleus of the stria terminalis (BnST) (Silverman *et al.*, 1981; Forray and Gysling, 2004; Silverman *et al.*, 2008). In addition to other hypothalamic and limbic inputs, the PVN also receives NA and 5HT projections from the brainstem (Forray and Gysling, 2004). Thus, it is very possible that the PVN plays a key role in integrating and coordinating autonomic, behavioural and neuroendocrine responses to stress (Champagne *et al.*, 1998; Tsigos and Chrousos, 2002).

1.2.1.2 Feedback mechanism of the HPA axis

There is a plethora of literature that detail the complex feedback mechanisms involved in the regulation of the HPA axis. Regulation of the HPA axis is predominantly mediated by CORT through receptors located in various brain areas especially the hypothalamus, anterior pituitary and hippocampus (Wilson *et al.*, 1980; Keller-Wood and Dallman, 1984; McEwen *et al.*, 1986; De Kloet and Reul, 1987; Jacobson and Sapolsky, 1991; Herman and Cullinan, 1997; Sapolsky *et al.*, 2002b).

Brain cells contain two types of corticosteroid receptors: type 1 or Mineralocorticoid Receptors (MRs) and type 2 Glucocorticoid Receptors (GR), originally classified during the 1960s (McEwen *et al.*, 1968; Gerlach and

McEwen, 1972; Reul and Kloet, 1985). These two receptor subtypes lie intracellularly and act as ligand activated transcription factors. MRs display a very high affinity for endogenous glucocorticoids (cortisol and corticosterone), whereas GRs have lower affinity for cortisol and corticosterone (Fink, 2000; Seckl, 2004; de Kloet *et al.*, 2005b; Maccari and Morley-Fletcher, 2007).

MRs are more specifically located than GRs which are scattered throughout the CNS and are present in both neurons and glial cells. MRs have limited distribution in the periphery (kidney, lymphocytes, colon) and in the limbic system of the brain, whereas GRs have ubiquitous expression in brain and periphery (Fink, 2000).

Meijer *et al.* (2007) found that MRs are largely found in the limbic system including the hypothalamus, the amygdala and dentate gyrus and pyramidal cells of the hippocampus: Both GRs and MRs are expressed by hippocampal neurones (Table 1.1) (Meijer *et al.*, 2007).

Table 1.1: Properties of Mineralocorticoid and Glucocorticoid receptors in the brain.

Receptors	Affinity	Locations	Examples
Mineralocorticoid receptors (MR) (type 1)	High affinity	Limbic system (hippocampus)	Cortisol, Corticosterone (aldosterone)
Glucocorticoid receptors (GR) (type 2)	Low affinity	Ubiquitous	Cortisol, Corticosterone (dexamethasone, RU 486)

Joels *et al.* (2007) suggested that MR activation is required to maintain neuronal integrity and a stable excitatory tone, at least in the hippocampus (Joëls *et al.*, 2007). Binding studies using hippocampal homogenates show that MRs have a 6-10-fold greater affinity for CORT in comparison to GRs (Reul and Kloet, 1985). Moreover, it is thought that this difference between the two receptor

subtypes in their affinity for CORT is responsible for the different level of occupancy of the receptor system of the hippocampus under basal conditions, and stress. In general, it appears that under basal conditions the MR population is almost 80% occupied as opposed to the 10-15% occupancy of GRs (Reul and Kloet, 1985; Reul *et al.*, 1987; Meaney *et al.*, 1988a).

It has been hypothesized that the tonic influences of corticosterone are exerted via hippocampal MRs (De Kloet *et al.*, 1998), while the additional occupancy of GRs with higher levels of corticosterone mediate feedback actions aimed at restoring disturbances in homeostasis (de Kloet and Reul, 1987). Under basal and stress conditions MR is involved in the regulation of the HPA axis activity in the CNS (de Kloet *et al.*, 1998); however they posit two modes of negative feedback: (1) Due to circadian rhythms "proactive" negative feedback mediated via the MR maintains normal variations; and (2) "reactive" feedback operating via the GR serves to inhibit further secretion of ACTH and facilitate the return to basal levels. Dallman *et al.* (1989) suggested that MR may be more important in regulating the basal expression of adrenocorticotropin factor (ACTH) and can cause secretion of CRF and AVP at the trough of diurnal ACTH secretion, and in the regulation of the nadir ACTH release.

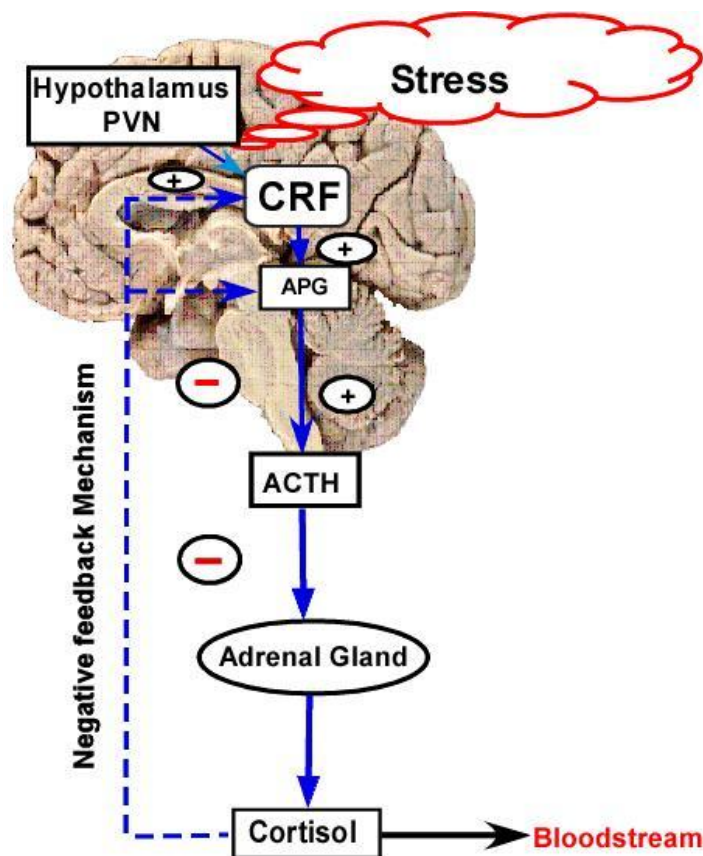


Figure 1-2: Hypothalamic-Pituitary-Adrenal (HPA) Axis.
 PVN=paraventricular nucleus; CRF=corticotropin releasing factor;
 ACTH=adrenocorticotrophic hormone; APG=anterior pituitary gland.

To maintain homeostatic balance, cortisol in low concentrations gives the PVN primarily positive feedback through MRs as well as terminating the axis' stress via negative feedback to the PVN and anterior pituitary through GRs (Steckler *et al.*, 2005) (Figure 1-2).

Corticosteroids acting through GRs have profound effects on energy metabolism; via GR corticosteroids activate psychological stress reactions, but inhibit inflammatory and immune responses, as well as the response of the HPA axis to stress. Importantly, GR activation generally derives energy sources from their catabolism of fat and proteins, ultimately liberating glucose for sustained metabolic responses i.e. gluconeogenesis. The principle MR is aldosterone – a

selectively acting steroid in other epithelial cells and kidney, which is involved in the regulation of electrolyte balance (De Kloet *et al.*, 2011).

For instance, low level CORT mediated activation of the MR enhances neuronal excitability e.g. long term potentiation (LTP) an effect that reverses with higher levels of CORT activity stimulating MR and GR (Joëls, 2008). Similarly, MR and GR appear to have opposite effects on anxiety modulation (Smythe *et al.*, 1997).

De Kloet *et al.* (1999) and Joels (2001) suggested that predominant MR activation, as opposed to concomitant activation of MR and GR together, can trigger distinct and even opposite responses in, for example, cognitive function and neuronal excitability (de Kloet *et al.*, 1999; Joels, 2001).

Regulation of CRF release is the primary target for CORT-mediated feedback upon HPA axis activity (Conrad, 2011). Studies have shown that the levels of CRF and AVP messenger ribonucleic acid (mRNA) expression in the parvocellular region of the PVN increase following adrenalectomy and thus in the absence of CORT (Herman *et al.*, 1995; Makino *et al.*, 1995; Herman *et al.*, 2008). This can be reversed to normal levels following CORT administration (Sawchenko *et al.*, 1984; Sawchenko, 1987; Beyer *et al.*, 1988; Sawchenko *et al.*, 1993). Interestingly, oxytocin (OXY) mRNA levels (Sawchenko and Arias, 2006) and pro-enkephalin levels are unaffected by adrenalectomy (Tanimura and Watts, 1998) thus highlighting the specificity of CORT-mediated negative feedback towards CRF and AVP. Seemingly, hypophysectomy also induces a rise in CRF and AVP transcripts in the parvocellular region of the PVN, suggesting a loss of ACTH-mediated negative feedback (Sawchenko and Arias,

2006). From these findings it is apparent that both CORT and ACTH mediate negative feedback on CRF release (Figure 1-2).

The hormones of the HPA axis play an important role in mediating the indirect regulation of activity through other brain areas in addition to direct feedback occurring within the HPA axis structures themselves. As a means of higher centre control, these regions provide an important neuronal input to the hypothalamus. For example, Gray and Bingaman (1996) demonstrated that a significant population of CRF neurones lie in the central nucleus of the amygdala (CeA). Stress responses in addition to autonomic activity may be modified through corticosteroids acting upon these neurones.

In contrast to reducing CRF mRNA expression in the PVN, CORT increases CRF mRNA expression in the CeA (Swanson and Simmons, 1989).

The regulatory influence of the hippocampus upon HPA function is well documented (Van Hartesveldt, 1975; Reul and Kloet, 1985; Herman *et al.*, 1989). Feldman and Conforti (1976) suggested that hippocampal stimulation inhibits both adrenocortical activity and the activity of PVN neurones projecting to the median eminence (Feldman and Conforti, 1976; Saphier and Feldman, 1987). Keller-Wood, (1996) also suggested that enhanced CORT feedback at the hippocampus negatively controls CRF and thus ACTH secretion. Furthermore, in the adult rat, hippocampal lesions or fornix transections lead to raised basal and stress-induced levels of CORT and ACTH, in addition to increased CRF and AVP mRNA. This indicates the loss of a site critical to CORT-mediated negative feedback (Knigge and Hays, 1963; Moberg *et al.*,

1971; Fischette *et al.*, 1980; Wilson *et al.*, 1980; Sapolsky *et al.*, 1984; Herman *et al.*, 1989; Sapolsky *et al.*, 2002a).

Landfield *et al.* (1978) clearly demonstrated the importance of the hippocampus in feedback control in the rodent, whereby the loss of hippocampal neurones with age corresponds with increasing CORT levels (Landfield *et al.*, 1978).

Sapolsky *et al.* (1986) reported that with the loss of neurones, aged rats have reduced numbers of hippocampal CORT receptors, corresponding with insensitivity to CORT-mediated negative feedback and a consequential hypersecretion of CORT both basally and in response to stress.

Clinically, CORT hypersecretion and associated negative feedback insensitivity has been associated with behavioural deficits in aged rats including cognitive effects (Landfield *et al.*, 1978; Sapolsky *et al.*, 1985; Sapolsky *et al.*, 2002b).

Corticosterone at high doses has the effect of reducing the size of cytosolic GR populations in the hippocampus (Tornello *et al.*, 1982; Tornello *et al.*, 2008). This is consistent with the effects of chronic stress and is associated with negative feedback (Herman *et al.*, 2008).

It has been suggested that sustained elevations of circulating CORT, either by repeated stress or exogenous administration significantly reduces CORT receptor populations in the hippocampus, the amygdala and frontal cortex (Sapolsky *et al.*, 1984; Tornello *et al.*, 1982; Tornello *et al.*, 2008), an effect not seen in the hypothalamus or pituitary. These findings provide strong evidence for a definitive role of these areas in mediating feedback upon HPA axis activity. Sapolsky *et al.* (1984) demonstrated that exogenous CORT administration has no greater effect in reducing receptor populations than stress alone, and thus

suggested the presence of a threshold. There is a notable reduction in GR levels one week following cessation of CORT treatment which shows the reversible properties of the receptors (Reul and Kloet, 1985; Reul *et al.*, 1987; Dallman *et al.*, 1989; Ratka *et al.*, 1989; Bradbury *et al.*, 1991; Reul *et al.*, 2008). There are long-term implications of CORT-mediated inhibitory effects upon the HPA axis due to the fluctuation of these receptor populations with CORT-mediated stimulation reducing and adrenalectomy increasing receptor populations accordingly (Sapolsky and McEwen, 1985; Reul *et al.*, 1987; Brinton, 1988; Luttge *et al.*, 1989).

1.2.2 PATHOPHYSIOLOGICAL CHANGES IN HYPOTHALAMO-ADRENAL-AXIS AXIS AND ITS RELATED CONSEQUENCES

Extensive research has focused on age-related abnormalities and the HPA axis (Meaney *et al.*, 1985; Seeman *et al.*, 2001; Heine *et al.*, 2004). In rodents, ageing has been associated with hypersecretion of CORT, occurring both basally and after stress (Sapolsky *et al.*, 1987; Scaccianoce *et al.*, 2008). This could be due to the loss of hippocampal neurones which has been shown to occur in aged rats (Landfield *et al.*, 1978; Sapolsky *et al.*, 1984; Sapolsky and Meaney, 1986; Scaccianoce *et al.*, 2008).

Studies have shown a reduction in the number of hippocampal neurons and a reduction in dendritic branching of the remaining neurones and impaired synaptic plasticity when rats are exposed to high CORT levels (Sapolsky, 1993; Bodnoff *et al.*, 1995; Sunanda *et al.*, 1995). In order to reduce hippocampal degeneration and attenuate cognitive impairments typically seen with ageing, it

has been suggested that less cumulative CORT exposure throughout life may preserve these effects (Meaney *et al.*, 1988a).

In humans, older men show a reduction in sensitivity of the HPA axis compared to younger men. Older men exhibit a slower response of ACTH to CORT-mediated feedback in comparison to younger men (Boscaro *et al.*, 1998; Wilkinson *et al.*, 2008). Further investigations have shown that hippocampal atrophy has been associated with traumatic stress, depression, ageing and Cushing's syndrome (Golomb *et al.*, 1994; Bremner *et al.*, 1995; Sheline *et al.*, 1996; Lupien *et al.*, 1998b; Starkman *et al.*, 1999).

The clinical relevance of HPA disturbances is that they are associated with psychiatric disorders, in particular hypocortisolism (Stokes and Sikes, 1991; Heim *et al.*, 2000). Hypocortisolism refers to a deficiency of cortisol, a reduction in adrenocortical secretion reactivity and enhanced negative feedback inhibition. This state has also been described in association with patients suffering from other disorders such as chronic fatigue syndrome and various stress-related syndromes such as Posttraumatic Stress Disorders (PTSD) (Demitrack *et al.*, 1991; Cleare *et al.*, 1995).

Two studies on chronic fatigue syndrome have found a possible therapeutic potential from hydrocortisone (Cleare *et al.*, 1999) and dehydroepiandrosterone (DHEA) administration (Scott *et al.*, 1999). Hypocortisolism has been reported to increase GR levels in lymphocytes (Yehuda *et al.*, 1993) and increase CSF concentrations of CRF (Bremner *et al.*, 1997). These findings are strikingly different to those associated with hypercortisolism along with other HPA axis

alterations, which have been recognised in depression (for review, see (Nemeroff, 1996).

The impact of chronic stress on the HPA axis and sympathetic systems leading to hypercortisolism may also lead to factors such as anxiety, feelings of hopelessness and depression (Chrousos, 1992).

With regard to the latter affective disorder, many studies have focused on its association with underlying HPA dysfunction. In general, the HPA axis abnormalities which commonly accompany depression represent a failure of stress adaptive processes (Chrousos GP, 1988), often expressed as raised HPA axis activity (Maes *et al.*, 1991).

In humans, major depression is frequently associated with elevated circulating CORT and unpaired CORT feedback (Kalin *et al.*, 1982; Evans *et al.*, 1983; Murphy, 1991). Hence, the dexamethasone suppression test, a quantifier of delayed CORT feedback at the pituitary level has been adopted as a diagnostic tool to assess depressed patients (Carroll, 1982; Checkley, 1996).

Feedback abnormalities of chronically-stressed animals and those of patients with depression bear some resemblance, such as enlargement of the pituitary and adrenal glands (Young *et al.*, 1991; Nemeroff *et al.*, 1992).

HPA axis disturbances and its role in CORT alterations are also evident at the hypothalamic level. Depressed patients have been found to hypersecrete CRF (Gold *et al.*, 1984; Roy *et al.*, 1987; Owens and Nemeroff, 1993), have reduced density of CRF binding sites in the frontal cortex (Nemeroff *et al.*, 1988) and a blunted ACTH response to CRF (Holsboer *et al.*, 1984; Amsterdam Jd, 1987; Arborelius *et al.*, 1999).

The exact relationship between HPA axis dysfunction and depressive symptomatology remains uncertain. There are similarities between chronic stress-mediated alterations to HPA activity and changes accompanying depression (Charmandari *et al.*, 2005), yet it has not been established which is the cause and which is the effect, or whether are both develop simultaneously.

1.2.3 STRESS AND NEURODEGENERATIVE DISORDERS

Hypercortisolemia and hippocampal atrophy are associated with Alzheimer's disease (AD) during old age. The role of stress in memory dysfunction and AD pathology has logically attracted some attention. According to the glucocorticoid cascade hypothesis, the loss of normal negative hippocampal feedback to the HPA axis could lead to increased cortisol secretion and hippocampal degeneration in AD. It is potentially a vicious circle as an overactive HPA system in AD could lead to additional neuronal degeneration thereby inducing progressive cognitive impairment (Davis *et al.*, 1986; Sapolsky *et al.*, 2002a; Pomara *et al.*, 2003).

Researchers suggest that persistent HPA activation and high cortisol levels could lead to memory dysfunction. This is one of the major clinical findings in Cushing's disease (CD), Parkinson's disease (PD) and major depressive disorders (MDD) (Lupien *et al.*, 1998b; Lupien *et al.*, 1999; Fink, 2000).

Women with a history of recurrent major depression have smaller hippocampal volumes; this was correlated with a lifetime of depression. The study also found that they did poorly on verbal memory tests (Lupien *et al.*, 1999; Sheline *et al.*, 1999; Fink, 2000).

In Parkinson's disease (PD), it is widely accepted that elevated cortisol levels result from abnormalities of the function of the HPA axis, possibly contributing to loss of dopaminergic neurons in the substantia nigra by a variety of mechanisms (Drevets, 2000; Pålhagen, 2009). These include excitotoxic cell death, increased intracellular calcium levels and increased oxidative stress (Dong *et al.*, 2009). However, more research is required in order to characterise and understand the contributions of stress in Parkinson's disease (PD) and with other neurodegenerative disorders (Fink, 2000).

Prolonged HPA overactivity is associated with cognitive decline and hippocampal atrophy in rodents (McEwen and Sapolsky, 1995; Lupien *et al.*, 1998a; McEwen, 1999). Rodents exposed to stress paradigms or administered large doses of corticosterone have been reported to have atrophy of the hippocampal neurons and an increased sensitivity to toxins. These findings however were not replicated in pigtailed macaque monkeys exposed to high doses of cortisone for a period of 12 months (de Kloet *et al.*, 1999; Koob and Heinrichs, 1999; Leverenz *et al.*, 1999; Pryce *et al.*, 2002; Tamashiro *et al.*, 2005). Cross-species differences may be an important factor as these primates in particular have proportionately fewer hippocampal GRs compared to rodents (Fink, 2000, Seeman *et al.*, 1997; Sapolsky *et al.*, 2002, de Kloet *et al.*, 2011; De Kloet *et al.*, 1998, Reul *et al.*, 1987).

1.2.4 STRESS AND EARLY LIFE EXPERIENCE

Early stressful life experiences are related to changes in behaviour, physiological responses to stress and may heighten the capacity to develop

psychopathology in adulthood (Heim and Nemeroff, 2001). For example, Maternal Separation (MS) is a well-characterized model of early life stress in rodents (Levine, 2000; Sanchez *et al.*, 2001; Newport *et al.*, 2002a). Long-lasting MS results in activation of the HPA axis during the stress hyporesponsive period (Huot *et al.*, 2002), a neonatal time characterised by weakened glucocorticoid responses to most adult stressors (Sapolsky and Meaney, 1986). Interestingly, long-lasting MS during the early postnatal period does not affect basal measures of HPA function in adulthood but instead causes discrete differences in the regulation of the HPA axis that emerge after exposure to stressors. Rodents exposed to extensively long periods of MS show increases in CRF, ACTH and CORT levels (Plotsky and Meaney, 1993; Liu *et al.*, 2000a).

In contrast, short periods of MS (e.g. neonatal handling (NH)) results in reduced CRF, mRNA, ACTH and CORT in response to stress (Meaney *et al.*, 1989; Plotsky and Meaney, 1993; Viau *et al.*, 1993; Liu *et al.*, 2000a). In addition to these hormonal changes, MS and NH are correlated with behavioural differences in later life. MS increases measures of anxiety (Huot *et al.*, 2001), debilitates maternal care (Lovic *et al.*, 2001) and diminishes spatial navigation learning (Huot *et al.*, 2002).

McCauley *et al.* (1997) in a human study on approximately 2,000 women, found that women with no history of childhood abuse showed fewer symptoms associated with depression and anxiety in comparison to women who had a history of physical or childhood sexual abuse. These women also had a higher frequency of suicide attempts (McCauley *et al.*, 1997). Moreover, suicide victims

in general have larger adrenal glands and low prefrontal cortex levels of corticotropin releasing binding protein consistent with a hyperactive HPA axis (Mann *et al.*, 1999; Fink, 2000; Mann, 2003; Oquendo Ma and *et al.*, 2003).

It has also been reported that parental loss, along with other childhood adversities are related to the development of depression and anxiety disorders in adulthood (Kendler *et al.*, 1992; Kessler and Magee, 1993; Agid *et al.*, 1999).

Adverse events during childhood have been reported to predispose individuals to the developing of post-traumatic stress disorder (PTSD) in response to extreme trauma (Bremner *et al.*, 1993; Zaidi and Foy, 1994). In addition to early-life trauma, stress in adulthood can also contribute to the expression of major depression and anxiety disorders, including PTSD (Read *et al.*, 2005; McEwen, 2008).

1.2.5 THE DEVELOPMENTAL PROFILE OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

Hormonal and receptor levels fluctuate greatly throughout the perinatal period with relation to the HPA axis. Sapolsky and Meaney (1986) have extensively reviewed these perinatal developmental changes (Sapolsky and Meaney, 1986). There is initial activity of the foetal rat HPA axis followed by a period of inactivity known as the stress hyporesponsive period. During this time there is reduced adrenal responsiveness to ACTH reduced, levels of CORT (Levine *et al.*, 1967; Guillet *et al.*, 1980; Guillet and Michaelson, 2008) and low hypothalamic levels of CRF (Bugnon *et al.*, 1984).

Over the succeeding period (postnatal days 2-7), levels of ACTH, CORT and CRF increase, and achieve adult levels by the third or fourth week postnatally (POMC) levels (Grino *et al.*, 1989; Sakly and Koch, 2008).

Analysis of glucocorticoid (GR) and mineralocorticoid (MR) receptors show that in the last week of gestation forebrain GR densities are comparable to those of the adult rat (Meaney *et al.*, 1985; Meaney *et al.*, 2008). This is in contrast to the first week of life, where the level of GR receptor binding in the rat brain is approximately 30% those of adult values in regions such as the hypothalamus, hippocampus and cortex (Meaney *et al.*, 1985; Rosenfeld *et al.*, 1988; Sarrieau *et al.*, 1988; Meaney *et al.*, 2008). The hippocampus receptor densities decline during the second postnatal week (Rosenfeld *et al.*, 1988; Rosenfeld *et al.*, 1992). In week three the GR levels increase to those of the adult suggesting the rat brain, especially the forebrain, is less sensitive to CORT for a 3-week postnatal period. It has been observed that GR binding patterns in the lung, intestine and pituitary are higher in contrast to regional development when compared to the adult during the first week of life (Henning *et al.*, 1975; Olpe and McEwen, 1976; Sakly and Koch, 1981; Kalimi, 1984; Sakly and Koch, 2008).

These disparities may highlight the functional involvement of GR activation during this 3-week period; for instance the importance of CORT in the induction of lung surfactant production. The same ontogenetic developmental pattern is followed by the plasma free CORT regulator corticotrophin binding globulin (CBG) and it achieves adult levels during the last week of gestation. CBG then decreases to a very low level at birth before requiring adult levels by the second

or third week postnatally (Henning, 1978; Smith and Hammond, 1991; Takahashi, 1998; Henning *et al.*, 2009).

In comparison to GRs, the development of MRs does not vary during the early postnatal period. The hippocampus shows a similar pattern for MR as for GR and no differences in mRNA levels for MR and GR between postnatal days two to four (Van Eekelen *et al.*, 1991; Lawson *et al.*, 1992).

1.2.5.1 HPA axis activity during pregnancy

Wasser (1999) suggested that social and psychological stressors have great influence upon reproductive success. Studies have attempted to identify the relationship between pregnancy and the role of the HPA axis along with others associated hormones. However, complications arise due to determining changes in HPA activity through gestation; both the placenta and foetus contribute to circulating hormone levels. Therefore, it is subject to debate regarding the hormones' origins and its derivation. During early gestation, there is a decrease in plasma CORT levels in rats (Ogle and Kitay, 1977; Atkinson and Waddell, 1995; Johnstone *et al.*, 2000; Brunton *et al.*, 2008).

Pregnancy represents a state of mild but sustained hypercortisolism, with maternal plasma CORT levels increasing during the second trimester until term in both rodents and humans (Carr *et al.*, 1981; Okamoto *et al.*, 1989; Waddell and Atkinson, 1994; Johnstone *et al.*, 2000).

Okamoto *et al.* (1989) reported that human infants showed a plasma ACTH rise after 16-20 weeks, reaching a peak at delivery (Okamoto *et al.*, 1989). Alterations in the relationship between CORT and ACTH also occur during this period. These changes occur due to alterations in sensitivity of the adrenal

glands to ACTH or reduced metabolic clearance of CORT (Waddell and Atkinson, 1994). Furthermore, there could also be a contribution from foetal adrenal glands (Dupont *et al.*, 1991). These data highlight the difficulty in establishing the actual status of the HPA axis and how its function changes during gestation and ensuing parturition.

Welberg and Seckl (2001) reported that the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) ameliorated the influence of prenatal CORT administration on foetal HPA activity (Welberg and Seckl, 2001). This enzyme exists in two isoforms and is prevalent within a variety of tissues including the placenta (Monder, 1991; Brown *et al.*, 1993; Lakshmi *et al.*, 1993). The enzyme catalyses the conversion of CORT (Cortisol in humans) to 11-dehydro CORT (Cortisone), the latter form being relatively inert in its actions. The type II form (11 β -HSD-2) is of interest due to its localisation in placental tissues (Waddell *et al.*, 1998) and the possibility that this protects foetal tissues against maternal CORT influences (Meyer, 1985).

During gestation the adaptation of the HPA axis occurs at many different levels, including changes in limbic feedback (Johnstone H.A, 1997), CRF and AVP hypothalamic neurones (Douglas and Russell, 1994), and pituitary and adrenal gland (Carr *et al.*, 1981; Waddell and Atkinson, 1994). In response to CORT, CRF and ACTH secretion is reduced while the adrenal gland shows an increase in sensitivity to ACTH and consequent CORT secretion (Dupouy *et al.*, 1975; Carr *et al.*, 1981; Waddell and Atkinson, 1994; Keller-Wood, 1996).

There appears to be an alteration in excitability and adaptation of the HPA axis (De Kloet *et al.*, 2005a). Throughout the third trimester in rats there is a rise in

both CORT and ACTH which then attenuates (Williams *et al.*, 1999). Since CRF-stimulated ACTH secretion is reduced with the advance of pregnancy and lactation, this blunting of the HPA response to stress may be due to physiological adaptations at the pituitary level (Magiakou *et al.*, 1996; Mastorakos and Ilias, 2003).

Neumann *et al.* (1998) reported a significant reduction in CRF receptor binding in the pituitary on gestational day 11 which is further reduced by gestational day 22 (Neumann *et al.*, 1998). On day 21 of pregnancy reduced responses to stress alongside reductions in PVN CRF and mRNA expression have been shown (Douglas and Russell, 1994; Neumann *et al.*, 1998).

In view of the data, it has been suggested that the reduced HPA responsiveness to stressors may be a protective mechanism for both the pregnant rat and its offspring against maternal CORT concentrations (Weinstock, 1997).

1.3 PRENATAL MANIPULATIONS: NEUROCHEMICAL AND BEHAVIOURAL RESPONSES OF OFFSPRING AND MOTHERS

1.3.1 IMPACT OF PRENATAL STRESS ON DEVELOPMENT

Behavioural development of offspring may be adversely affected by increased HPA activity during pregnancy. For example, adult monkeys and rodent's show reduced abilities to cope under stress when subjected to prenatal stress. The related HPA dysfunction manifests as a prolonged rise in stress-induced plasma CORT and reduced feedback inhibition of CRF.

Weinstock (1997) highlighted that these effects are thought to be programmed into the offspring's physiology by exposure to high levels of maternal hormones during gestation (Weinstock, 1997). These alterations may affect offspring making them more vulnerable to physiological and psychological disturbances in later life.

In general, pregnant rats exposed to stress gain less weight in pregnancy, give birth earlier and have fewer progeny compared to non-stressed controls (Barlow *et al*, 1978; Fride and Weinstock, 1984; Williams *et al*, 1998).

Furthermore, a significant positive correlation between infant morbidity and maternal stress has been found (Stott, 1973; Jones and Tauscher, 1978; Cohen *et al*., 1980; Turner *et al*., 2005). Studies by Sackett (1990) in monkeys showed that while gestational stress is associated with increased foetal loss (Sackett, 1990), the surviving offspring exhibit an increased prevalence of cognitive deficits (Sackett, 1991).

Jones and Tauscher (1978) reported that in humans, psychological stress during pregnancy correlates with an increased likelihood of the offspring suffering from congenital malformations and low birth weight (Jones and Tauscher, 1978). Prenatal Stress (PS) has been associated with developmental delays, cognitive impairments and altered emotionality.

Retrospective analysis of emotionally disturbed youths and their maternal background indicates that there is a strong link between both physiological and psychological risk factors to later development of childhood psychopathology and chronic stress experienced by the mother during pregnancy (Ward, 1991). Similar retrospective studies have found associations between PS and

depression, schizophrenia, psychosis, hyperactivity and alcoholism (Huttunen and Niskanen, 1978; Brixey *et al.*, 1993; Weinstock, 1997).

1.3.2 PRENATAL STRESS AND HYPOTHALAMO-PITUITARY-ADRENAL AXIS CHANGES

Many studies show that disturbances in HPA axis function follow from PS-induced manipulations (Weinstock *et al.*, 1988; Henry *et al.*, 1994a; Barbazanges *et al.*, 1996b; Zagron and Weinstock, 2006).

For example, maternal CRF administration during the third trimester reduces pup weights, increases the anogenital distance of male progeny and increases ultrasonic vocalizations (USVs) of pups emitted during isolation (Williams and Russell, 1972; Williams *et al.*, 1995; Williams *et al.*, 1998b).

It has been shown that prolonged gestation, abnormal development of the young and impaired onset of maternal behaviour occur following daily treatment with ACTH during the third trimester (Fameli *et al.*, 1995).

Fride *et al.* (1986) reported that the offspring of pregnant rats exposed to chronic stress had significantly higher levels of plasma CORT compared to non-stressed controls. These increased levels of CORT impact foetal adrenal function since maternal CORT reaches the foetal bloodstream through the placenta (Zarrow *et al.*, 1970; Henry *et al.*, 1995). Hence, the effects of PS on the offspring are partly mediated via placental hormone transfer (Joffe and Joffe, 1978; Kapoor *et al.*, 2006).

Observations of infant primates show that the primate HPA axis is not mature until after birth (Ducsay *et al.*, 1991) yet the foetal primate HPA axis does respond to exogenous hormonal stimulation by mid-gestation (Ducsay *et al.*,

1991; Nguyen and Conley, 2008). It has been reported that such vulnerability to external activation is readily apparent in the offspring of monkeys who were psychologically stressed through mid-to-late gestation and were shown to have higher basal ACTH and CORT levels in comparison to controls and exhibit higher ACTH levels during stress (Clarke *et al.*, 1994).

It has been established that maternal stress experienced throughout gestation influences foetal HPA development (Welberg and Seckl, 2001). The overall effect of PS is to elevate HPA axis reactivity of the offspring, the manifestations of which are seen in the very early stages of life.

Following prenatal stress, (e.g. footshock), every other day during gestation produces offspring which, exhibit significantly higher basal levels of ACTH and CORT in comparison to controls as early on as postnatal day one (Takahashi and Kalin, 1991). Moreover, 14 day old offspring of prenatally-stressed dams, also exhibit increased ACTH and CORT responses to footshock (Takahashi *et al.*, 1988; Takahashi and Kalin, 1991), but emit fewer USV's in response to isolation (Takahashi *et al.*, 1990).

On further investigation, by postnatal day 14 it can be seen in both prenatally-stressed and control offspring that CORT/ACTH significant increases in response to footshock stress and similar patterns of decline, although prenatally-stressed rats' show greater magnitude responses than the controls (Takahashi and Kalin, 1991). This difference was not observed when the offspring were assessed on postnatal day 21.

Takahashi *et al.* (1992a) reported no differences amongst stressed/control progeny as adults, although others researchers have shown that prenatally-

stressed rats exhibit increased basal and stress-induced plasma ACTH (Takahashi *et al.*, 1992a; McCormick *et al.*, 1995). In addition, compared to the controls, stressed progeny show prolonged stress-induced CORT secretion (Peters, 1982b; Fride *et al.*, 1986b; Weinstock *et al.*, 1992; Henry *et al.*, 1994b; Maccari *et al.*, 1995a; McCormick *et al.*, 1995).

Various theories suggest the mechanisms by which programming of foetal HPA activity takes place. Most importantly, the release of maternal pituitary-adrenal hormones in response to stress has been suggested to downregulate GRs and/or cause degeneration of hippocampal cells in the progeny (Sapolsky *et al.*, 1987; Sapolsky *et al.*, 1990). This, in turn, may alter infant HPA reactivity through an impairment of negative feedback (Sapolsky *et al.*, 1984; Fride *et al.*, 1986a; Sapolsky *et al.*, 1990). Similar findings have been reported in previous investigations of stressed offspring where these impairments such as prolonged elevated secretion of CRF and CORT have been found (Weinstock, 1997). In agreement other reports showing that blocking the mothers' stress-induced CORT secretion leads to a suppression of both stress-induced CORT hypersecretion and a reduction in hippocampal GRs in adult offspring of stressed rats (Barbazanges *et al.*, 1996a).

The hippocampus is vulnerable to damage during early development and the binding capacity of hippocampal CORT receptors is a primary regulating factor in subsequent CORT secretion (De Kloet and Reul, 1987; McEwen *et al.*, 1986). Thus, the findings by Maccari *et al.* (1995) show that the reduced binding capacity of hippocampal CORT receptors in prenatally-stressed adult rats (Maccari *et al.*, 1995) represents one possible mechanism via which such

altered HPA axis activity in the offspring is mediated. Interestingly, the MRs of the hippocampus are thought to be responsible for maintaining basal CORT activity.

It has been identified that a reduction in population size of MR may convey disturbances in basal levels of CORT following prenatal stress. Whether hypothalamic mechanisms controlling CORT are affected by PS remains unclear. Smythe *et al.* (1996) found that changes in CRF content in the median eminence following PS were dependent upon the early postnatal environment (Smythe *et al.*, 1996).

1.3.3 PRENATAL STRESS AND CENTRAL AMINERGIC SYSTEMS

It has been well documented that PS affects the serotonergic system (Peters, 1982b; Peters, 1986a; Peters, 1986b; Peters, 1990). Prenatal stress also affects other brain neurochemical systems including catecholamines and opioid systems (Peters, 1982a; Fride *et al.*, 1985; Fride and Weinstock, 1989; Insel *et al.*, 1990; Peters, 1990; Takahashi *et al.*, 1992b; Alonso *et al.*, 1994), which in later life may indirectly affect behaviour and stress responses. For example, PS reduces the density of benzodiazepine receptors in the hippocampus, which act as an inhibitory in effect upon the stress response (Fride *et al.*, 1985; Weinstock, 2007).

Serotonin plays an important role in the regulation of the HPA axis (Mitchell *et al.*, 1990; Tsigos and Chrousos, 2002) and in early brain development (Lauder and Krebs, 2010).

Stress exposure during gestation increases levels of 5HT and its primary metabolite 5-hydroxy-indole acetic acid (5HIAA) and tryptophan as pre-cursor in

the mother, in addition to increasing foetal brain tryptophan synthesis (Knott and Curzon, 1972; Peters, 1986a; Muneoka *et al.*, 1997). Moreover, the brains of prenatally-stressed adult rats have been shown to contain reduced levels of 5HT in conjunction with raised 5HIAA, indicating elevated 5HT metabolism (Hayashi *et al.*, 1998).

Animals also display persistent alterations in 5HT receptor binding, with increases in cortex, reductions in the hippocampus and normal binding in the pons and medulla (Peters, 1986a).

Matsukawa *et al.* (1997) have suggested that serotonin is implicated in the facilitation of synapse formation and maintenance (Okado *et al.*, 1993; Matsukawa *et al.*, 1997). This concept together with the above findings may offer some explanation as to the mechanisms responsible for the significant reduction in hippocampal synaptic density found to occur in prenatally-stressed rats (Hayashi *et al.*, 1998).

Rats prenatally exposed to ACTH have reduced dopamine (DA) activity in addition to increased 5HT activity (Fameli *et al.*, 1994), notably within the medial preoptic nucleus (Fride *et al.*, 1985), a region specifically associated with sexual differentiation. One of the many behaviours which PS appears to disrupt is sexual differentiation and sexual activation. DA levels are reduced in conjunction with increased levels of DA metabolites, indicative of elevated turnover, have been found in the ventral tegmental area (VTA) of stressed animals, in parallel to raised NA turnover in the LC (Takahashi *et al.*, 1992b).

Noradrenergic cells of the LC innervate the hippocampus and increase NA release in the hippocampus following stress (Tanaka *et al.*, 1983; 1991).

Moreover, it has been identified that in addition to MR and GR mRNA, a large majority of cells in the cornu ammonis fields and dentate granule cell layers express $\alpha 1$ - receptors (Williams *et al.*, 1997).

Lesioning of these ascending noradrenergic pathways have been shown to increase hippocampal CORT binding and attenuate stress-induced CORT secretion (Maccari *et al.*, 1990). This suggests that CORT receptors may be regulated via the noradrenergic system (Blanchard *et al.*, 2001).

The increased manifestation of stress-induced behaviours may be partly explained by increased catecholamine turnover following prenatally-stressed and it may, therefore be associated with the regulation of hippocampal CORT receptor populations (Takahashi, 1998; Koehl *et al.*, 1999). It may represent a key pathway by which PS increases the behavioural responsiveness to stress, evident in early life and continuing into adulthood in the offspring of stressed rats (Fumagalli *et al.*, 2007; Wright *et al.*, 2007).

1.3.4 PRENATAL STRESS AND BEHAVIOUR

Several studies have investigated the effects of prenatal stress upon behaviour. One of the earliest studies was by Thompson (1957), who found that there was a reduced level of exploration in an open field procedure in progeny of prenatally-stressed rats (Thompson, 1957). This finding has since been reproduced (Hockman, 1961; Fride *et al.*, 1986b; Wakshlak and Weinstock, 1990) alongside similar findings such as increased defecation (Thompson, 1957; Wakshlak and Weinstock, 1990).

The data supports the view that prenatally-stressed rats exhibit heightened emotionality like-behaviour during development and adulthood (Thompson, 1957; Hockman, 1961; Archer and Blackman, 1971; Peters, 1982a; Weinstock *et al.*, 1988; Vallée *et al.*, 1997). However, reduced emotionality like-behaviour has been observed by using dams stressed during pregnancy that did not postnatally change the emotionality like-behaviour of non-prenatally stressed offspring when reared. However, the reasons for this may be that the prenatal stress during gestation period is inadequate by itself to change the offspring's emotionality like-behaviour.

It is suggested that the direction of changes occurred in offspring behaviour is probably dependent on the magnitude of stress and the trimester during pregnancy at which it was administered (Thompson, 1957; Hockman, 1961; Chapman and Stern, 1979; Huizink *et al.*, 2004).

Prenatally-stressed offspring also exhibit behavioural disturbances such as suppressed social behaviour (Dunn and File, 1987; Takahashi *et al.*, 1992a) and raised anxiety like-behaviour in novel environments such as, the Elevated Plus maze (Fride and Weinstock, 1988; Dunn and Berridge, 1990; Vallée *et al.*, 1997). Interestingly, primates display general behavioural alterations in response to PS. It was found that such offspring demonstrate more abnormal social behaviour such as mutual clinging and reduced proximity and contact, when compared to non-stressed controls (Clarke and Schneider, 1993).

These findings suggest that social behaviours are more affected than non-social ones and that enhanced responsiveness to stressors may occur in later life, showing agreement with the rodent findings (Weinstock, 1997).

Retarded motor development is another important finding that has been identified within the progeny of prenatally-stressed animals (Fride *et al.*, 1986). They are generally found to be less active motorically. In addition to being less active, prenatally-stressed rats exhibited significantly raised CORT levels following each exposure to an open field from birth to postnatal day 8. This latter finding offers a link between changes in HPA axis activity and behaviour. It has been reported that many of the behavioural alterations seen in prenatally-stressed offspring are similar to those of adult rats following intracerebroventricular administration of CRF (Ward, 1972a; Weinstock *et al.*, 1988).

In tandem with such findings, adrenalectomy attenuates some behavioural effects of PS (Weinstock, 1997). Evidently the HPA axis is involved in the behavioural changes manifested in prenatally-stressed progeny (Fujioka *et al.*, 2001; Bosch *et al.*, 2007).

1.3.5 PRENATAL STRESS AND SEXUAL BEHAVIOUR

It has been well documented that there are recognised differences between the sexes in terms of PS-induced effects on sexual behaviour. Male offspring of dams having undergone gestational stress display a feminisation of sexual behaviour and incomplete masculinity (Ward, 1972b; Ward, 1984; Anderson *et al.*, 1986; Ward, 1991; Ward and Stehm, 1991). Prenatal stress promotes maternal behaviour in male rats towards neonates (Ward, 1972a; Gué *et al.*, 2004), with males displaying full maternal behaviour faster than females (Kinsley and Bridges, 1988; Lonstein and De Vries, 2000).

The differential effects of PS upon sexualisation and associated behaviours appear to be reflected in other behaviours involving motoric performance and emotionality (Lonstein and De Vries, 2000).

In active avoidance tasks PS appears to suppress the performance of males, but facilitates the performance of females (Fride *et al*, 1986). However, the sex differences seen in open field behaviour, where females are usually more active than males, has been shown to be unaffected by PS (Alonso *et al.*, 1991b; Palanza, 2001).

The enhanced performance of prenatally-stressed males compared with controls in a water maze task, is thought to be related to raised emotionality and a heightened response to stress, rather than effects on cognition (Szuran *et al.*, 1994).

Furthermore, PS reduces sex differences reported for depressive tasks such as, swim tasks where males normally exhibit greater immobility during a forced swim task than females (Alonso *et al.*, 1991a; Palanza, 2001).

These latter findings together with those of Alonso *et al.* (1991b) with regard to open field behaviour show that the effect of PS on sex differences in behaviour are dependent on the nature of the behaviour (Alonso *et al.*, 1991b). The authors suggest that sex differences in models of depression may possibly be under the influence of gonadal steroids during prenatal development (Alonso *et al.*, 1991b; Palanza, 2001).

1.4 POSTNATAL MANIPULATIONS: NEUROCHEMICAL AND BEHAVIOURAL RESPONSES IN MOTHERS AND OFFSPRING

1.4.1 POSTNATAL ENVIRONMENT AND THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

Environmental conditions in early in life can impose long-lasting effects upon HPA axis regulation (Levine, 1962; Levine *et al.*, 1967; Meaney *et al.*, 1988b; Meaney *et al.*, 1989; Plotsky and Meaney, 1993; Ladd *et al.*, 1996). Evidence suggests that prenatal and postnatal manipulations such as PS, neonatal handling and maternal deprivation produce effects at various levels of the HPA axis that persist long into adulthood (Maccari *et al.*, 1995a; Meaney *et al.*, 1996b).

Maternal deprivation is thought to be the most potent naturalistic stressor to which rats can be exposed during the first two postnatal weeks (Stanton *et al.*, 1988; Kuhn *et al.*, 1990). This treatment imposed during the pre-weaning period for just one hour is sufficient to increase CORT release in pups (Kuhn *et al.*, 1990; Stanton *et al.*, 1988) and such an experience can increase HPA responses to stress in the offspring as adults if prolonged up to 24 hours (Plotsky and Meaney, 1993).

The effects of neonatal handling upon HPA axis programming contrast with maternal deprivation. Even though the standard animal care regiments such as routine cage changing produce a slight handling-like effect, it is not as powerful as the neonatal handling paradigm (Meaney *et al.*, 1991). The procedure developed by Ader and Grotta (1969) involves rat pups being briefly removed and isolated from their mothers for a 20 minute period on 1-14 of the postnatal period. Weinberg and Levine reported that neonatal handling has the effect of

modifying HPA responses well into adulthood (Weinberg and Levine, 1977; Weinstock *et al.*, 1992) and that these alterations remain apparent even at two years of age (Zarrow *et al.*, 1972; Meaney *et al.*, 1988b; Liu *et al.*, 1997a; Meaney, 2001). Rats exposed to such treatment display reduced ACTH and CORT responses to acute stress challenge (Hess *et al.*, 1969; Vallée *et al.*, 1997) and a faster recovery of these hormones to basal plasma levels following acute stress (Hess *et al.*, 1969; Ader, 1970; Weinberg and Levine, 1977; Freide and Weinstock, 1984; Wakshlak and Weinstock, 1990; Weinstock, 1997).

The rapid normalisation of basal HPA axis activity is believed to occur either through a strengthened response of CORT-mediated feedback (Levine, 1962; Meaney *et al.*, 1988) or by enhanced sensitivity to such feedback (Meaney *et al.*, 1989). In accordance with these changes in CORT and ACTH, studies showed that hypothalamic responses to stress are altered, with levels of CRF and AVP being lower than normal (Campbell *et al.*, 1973; Plotsky and Meaney, 1993; Liu *et al.*, 1997a). These handling effects upon HPA activity provide strong evidence that environmental conditions have a great influence upon early development and programming (Meaney, 2001; Weaver *et al.*, 2004; Zhang *et al.*, 2004).

Neonatal handling reduces hypothalamic CRF and AVP levels and thus causes reduced stimulation of ACTH release. Handling increases GRs in the hippocampus (Meaney and Aitken, 1985; Liu *et al.*, 1997b). It is reported that a significantly greater expression of GRs in the hippocampus and frontal cortex may be indicative of the mechanism by which enhanced suppression of post-

stress HPA activity occurs in these animals (Meaney *et al.*, 1989; Liu *et al.*, 1997a).

In handled rats both CORT and dexamethasone showed a more effective inhibition of ACTH responses to stress and the alterations to hippocampal GRs are consistent with increased negative feedback efficiency (Meaney *et al.*, 1989; O'Donnell *et al.*, 1994; Weinberg *et al.*, 1995; Liu *et al.*, 1997a).

Early handling has shown related behavioural effects such as reduction in anxiety and helplessness behaviour (Ferré *et al.*, 1995), increased nociceptive thresholds (Smythe *et al.*, 1994) and prevents age-related hippocampal neurone loss and memory impairment (Meaney *et al.*, 1991). The mechanisms involved in all of these receptors are yet to be fully understood.

1.4.2 MATERNAL CARE AND OFFSPRING DEVELOPMENT

Adverse early experience in humans, such as parental loss, inadequate care, neglect, or abuse, is often precedent in the later development of affective disorders (Harris *et al.*, 1990). For example, childhood abuse and neglect may lead to poor mental health including antisocial personality disorder (Luntz and Widom, 1994) and major depressive disorders (Hall *et al.*, 1993).

Coplan *et al.* (1996) suggested that such adverse early experience and associated psychopathology are related to disruption of hormonal and neurochemical systems (Coplan *et al.*, 1996).

This has been demonstrated using infant rhesus monkeys, which were exposed to more complex-environments, compared to less complex environments and exhibited diminished adrenocortical responses to stress (Clarke *et al.*, 1994).

In one study, infant monkeys displayed persistently raised levels of CSF CRF following exposure to adverse early rearing conditions which were thought to suggest differential effects upon extrahypothalamic CRF systems and the HPA axis (Coplan *et al.*, 1996). Interestingly, these findings suggest that hyperactivity of CRF neurones is implicated in the pathophysiology of certain human affective and anxiety disorders (Gold *et al.*, 1984). Hence, CRF is thought to be a potential neurobiological mediator through which early life stress may contribute to adult psychopathology. The pathways by which such proposed CRF-mediated pathology occurs may or may not involve those in association with the HPA.

Perinatal manipulations where the effects persist into adulthood and which affect physiology and behaviour (Meaney *et al.*, 1989; Takahashi *et al.*, 1992a; McCormick *et al.*, 1995) often relate to mother-pup interactions (Moore, 1984; Power and Moore, 1986). Maternal behaviours such as nursing (Maccari *et al.*, 1995) and licking pups (Barbazanges *et al.*, 1996a) are altered and these alterations have been associated with changes of the HPA axis in the offspring. It has been reported that as adults, pups that receive more licking and grooming from their mothers in the first ten days of life have reduced plasma ACTH and CORT responses to stress. These changes occur in addition to raised hippocampal GR mRNA, enhanced CORT feedback sensitivity and reduced hypothalamic CRF mRNA (Liu *et al.*, 1997a).

Moreover, neonatal handling alters maternal care; for example, mothers of handled pups lick and groom their offspring more and demonstrate greater amounts of arched-back nursing compared to the mothers of non-handled pups.

Similar observations have been reported by Lee and Williams with altered mother-pup interactions following handling (Lee and Williams, 1974; Champagne *et al.*, 2004). The altered HPA axis mechanism of handled pups, be it a direct effect of the handling or as a result of handling-induced changes in maternal behaviour, has yet to be confirmed. However, it appears that individual differences in responses to stress are strongly related to maternal caregiving through such behaviours affecting the programming of HPA axis activity in offspring. Furthermore, it has been shown that this pattern of HPA axis activity may be passed to subsequent generations of offspring as an inheritable trait i.e. epigenetic (Francis *et al.*, 1999a; Weaver *et al.*, 2004; Weaver *et al.*, 2006).

The precise reason as to why patterns of maternal care may differ towards offspring remains unknown. Several studies have reported that pups that differ from one another may themselves elicit different patterns of care from the same dam (Ressler, 1962; Meier and Schutzman, 1968; Lee and Williams, 1974; Moore and Morelli, 1979; Macrì and Würbel, 2006; Tang *et al.*, 2006; Macrì *et al.*, 2008).

For example, handled pups produce more ultrasonic vocalisations (Bell *et al.*, 1971), possibly leading to increased maternal care. It has been reported that ultrasonic calls from pups have been associated with the initiation of anogenital licking by the dam (Brouette-Lahlou *et al.*, 1992; Hofer, 1996; Branchi *et al.*, 2001).

Moreover, prenatally-stressed pups elicit less maternal licking from non-stressed foster dams than do controls while stressed dams lick non-stressed foster pups less than do control pups (Moore and Power, 1986; Meaney, 2001;

Weinstock, 2008). Such studies reveal that the bond between a mother and her offspring is complex (Fleming *et al.*, 1999).

Apparently the biochemistry of the infant appears to be influenced by both the environment and the maternal care it received (Kalinichev *et al.*, 2002). Both factors are recognised as key for HPA axis function programming (Weaver *et al.*, 2004). Another key factor is the influence of the offspring toward maternal care and how infant behaviours may well elicit different levels, magnitude and type of maternal nurturance (Herbert *et al.*, 1982; Francis *et al.*, 1999b; Bornstein, 2002).

1.5 DEPRESSION

1.5.1 OVERVIEW

Depression has long been recognised and described in human history. However, it was Hippocrates who was the first to suggest that depression is due to physiological dysfunction. About 400BC Hippocrates argued that depression was due to an imbalance in the four humours: blood, phlegm, yellow bile and black bile, with an excess of melancholia or black bile causing depression (Sierra and Berrios, 1998).

More recently, Freud in his classic paper “Mourning and Melancholia”, discussed possible psychological causes for depression and noted that some depressions were clearly biological in aetiology (Freud, 1957). Over the past 3 decades, considerable progress has been made in understanding depressive disorders.

The signs and symptoms of depression are complicated and differ widely between people (Table 1-2).

Table1- 2: Signs and symptoms of depression.

Lethargy.
Sadness.
Loss of self-confidence and self-esteem.
Difficulty concentrating.
Not being comfortable in a group.
Feeling anxious all the time.
Avoiding people, sometimes even your close friends.
Feelings of hopelessness and helplessness.
Insomnia.
Very strong feelings of guilt or worthlessness.
Finding it hard to function at work/college/school.
Loss of appetite.
Loss of sex drive and/or sexual problems.
Physical aches and pains.
Suicidal tendency.
Self-harm.

1.5.2 DEPRESSION – FACTS AND FIGURES

Depression is one of the most prevalent psychiatric disorders affecting approximately 20-25% of women and 10-15% of men (Kessler *et al.*, 2005; Levinson, 2006). It is not certain why there are consistently higher figures for women in comparison to men. According to the longitudinal study on mental health of Adults in Great Britain by National Statistics in (2003) women are more likely to report symptoms of common mental health problems (Lewis, 2003). The National Institute for Health and Clinical Excellence (NICE) predicts that 9.8% of 16 to 65 year olds in the UK are suffering from mixed depression and

anxiety at any one time. 1 in 4 women will require treatment for depression at some time in their lives, compared to 1 in 10 men. The reasons for this are unclear, but are thought to be due to both social and biological factors. It has been suggested that depression in men may be under diagnosed because they present themselves to their general practitioner (GP) with different symptoms (National Institute For Clinical Excellence, 2003).

Depressive disorders are more common in urban than rural populations and, in general, the prevalence is higher in groups with adverse socio-economic factors (e.g., homeless people) (Lorant *et al.*, 2003).

Depression is recurrent with each episode increasing the risk of developing subsequent ones (Kessler, 2005). 1 in 5 people will not recover fully from their first episode and, in 70-80% of those reaching remission, depression will reoccur at least once (Kessler, 2005). This high reoccurrence rate seen in depression suggests that there are specific factors that serve to increase people's risk of developing repeated episodes of the disorder.

1.5.3 IMPACT OF DEPRESSION

The World Health Organisation (WHO) estimates that depression is the second leading cause of disability worldwide (Murray and Lopez, 1997). In 2007 the burden of depression was compared with that of chronic illnesses including angina, arthritis, asthma and diabetes. Depression was shown to have the largest effect on worsening health and individuals with depression comorbid (Moussavi *et al.*, 2007).

The economic burden of depression largely focuses on increased healthcare costs. Depression is associated with increased costs in every aspect of healthcare, including the costs of antidepressants and specialist mental health services. In the UK the cost of depression from working days lost and lost productivity is much higher than compared with its direct healthcare costs. In 2000 the total cost of depression to the economy was estimated to be over £9 billion, with only £370 million attributed to direct National Health Service (NHS) costs (Thomas and Morris, 2003).

The burden of ill health experienced by individuals with depression is associated with poorer physical health, especially high rates of cardiac problems and higher rates of smoking. Besides physical problems associated with depression, research indicates that individuals with depression have impaired social and work functioning, compared with non-depressed individuals (Hammen, 2005).

Depression adversely affects the quality of interpersonal relationships, in particular, relationships with children and spouses. Divorce rate is higher among depressed individuals than among non-depressed individuals (Wade and Cairney, 2000) and children of depressed parents are themselves found to be at increased risk of developing a related psychopathology (Goodman and Gotlib, 1999). The impact of depression on the individual's quality of life is reported to be directly related to the level of its symptom severity.

1.5.4 CAUSES OF DEPRESSION

The cause of depression remains a mystery even though researchers suggest various reasons for it e.g. unemployment, bereavement, adverse childhood experiences, family problems or other life changing events, or as a result of sudden physical illness (Beck and Alford, 2009).

There are a few examples of chronic illness that may be linked to depression including heart disease, spinal damage leading to back pain, renal disease and cancer. It has been clearly established the role of HPA axis in depression (Pariante and Lightman, 2008). Thus pituitary damage may also lead to depression (Beck and Alford, 2009).

Schatzberg and Rothschild (1992) reported that a variety of biological variables e.g. glucocorticoid activity, levels of dopamine, dopamine beta-hydroxylase activity and serotonin metabolites, have been examined in psychotic depression (Schatzberg and Rothschild, 1992). The secretion of cortisol is the most frequently studied measure and the method is most commonly used by dexamethasone suppression test (DST) (Carroll *et al.*, 1981; Nelson and Davis, 1997). Although an elevated level of cortisol has been reported in depression, other studies did not find a significant difference in rates of non-suppression of cortisol on the DST (Schatzberg, 1983; Evans and Nemeroff, 1987; Rothschild *et al.*, 1987).

Pariante (2006) reported that new evidence showing abnormalities in the GR play an important role in the pathophysiology of HPA axis hyperactivity in depression. The Mineralocorticoid Receptors (MR) have a high affinity for endogenous corticosteroids, while the GR have a high affinity for

dexamethasone and a lower affinity for endogenous corticosteroids (Pariante, 2006). The GR considered more substantial in the regulation of the response to stress when glucocorticoids endogenous levels are high in cases of depression (Pariante, 2006).

It found that a major depression is impaired of GR-mediated feedback inhibition, these came from other studies showing both expression in the GR and functional changes in those patients: administration of dexamethasone causes non-suppression in cortisol secretion; in vitro impaired GR function in peripheral blood mononuclear cells isolated and cultivated , or in vivo impaired in peripheral cells examined using vascular or metabolic indices; in postmortem human brains showed a reduction in GR expression in neuropathological studies (Pariante *et al.*, 2004; Juruena *et al.*, 2006; Pariante, 2006; Carvalho *et al.*, 2008; Carvalho and Pariante, 2008; Pariante and Lightman, 2008). Moreover, other ideas suggested that impaired GR function is crucial for HPA axis hyperactivity in depression, in laboratory animals and humans, antidepressant drugs have been shown to increase GR function, GR expression and GR-mediated HPA axis feedback inhibition, herewith, stimulated HPA axis and reducing resting activity. Finally, long-term clinical outcome, antidepressant drugs have been found to be a significant predictor of normalisation of GR function (Pariante *et al.*, 2004; Juruena *et al.*, 2006; Pariante, 2006; Carvalho *et al.*, 2008; Carvalho and Pariante, 2008; Pariante and Lightman, 2008).

Recently, Chourbaji et al. (2008) have shown that depressive-like behaviour occurs by decreasing of GR expression in experimental models (Chourbaji et al., 2008).

1.5.5 CLASSIFICATION OF DEPRESSION

The International Classification of Diseases (ICD) is the standard diagnostic tool for epidemiology, health management and clinical purposes (World Health, 1977). The International Classification of Disease (ICD-10) Classification of Mental and Behavioural Disorders, is the diagnostic framework used by clinicians in the United Kingdom (UK) to diagnose depression (All, 1995). The ICD-10 is a uni-axial system (World Health, 1992). The Diagnostic and Statistical Manual of Mental Diseases Fourth edition (DSM-IV) is a multi-axial system and forms the diagnostic framework used by clinicians in the United States of America (USA) to diagnose depression (American Psychiatric, 2000). DSM-IV is the most widely used diagnostic classification system in research, whereas ICD-10 is more widely used clinically (American Psychiatric, 2000 , All, 1995).

Various assessment scales are also used to assist in diagnosis, examples include; Hamilton Depression Rating Scale (HDRS) which is used to measure the symptoms of patients suffering from depression (Hamilton, 1960) and the Beck Depression Inventory (BDI), which is used to assess the intensity of the depression (Beck At, 1961).

1.5.6 RISK FACTORS FOR DEPRESSION

1.5.6.1 Genetic Factors

Parents, siblings and children of severely depressed patients have a higher lifetime risk of developing a mood disorder (10-15%) in comparison to the general population (1- 2%) (Mood, 1996). Two studies provide strong evidence that these may be a contributing factor in the development of depression (Kendler *et al.*, 1992; Kendler *et al.*, 1993; Kendler *et al.*, 1995; Kendler, 1998). Studies have shown that adopted children born to parents with a history of depressive disorder, but raised by adoptive parents with no such history have a higher risk of developing depressive disorder themselves, in comparison to a child who is born to parents with no history of depressive disorders (Levinson, 2006; O'Connell *et al.*, 2009).

Specific genes or series of genes have not been fully identified in the causation of depression; however, polymorphisms may increase the risk for later developing depression. Genes can predispose individuals to depressive disorder in many ways (Caspi and Moffitt, 2006; Levinson, 2006; Risch *et al.*, 2009).

1.5.6.2 Socio-economic Factors

The literature on SES provides strong evidence which indicates that social class and economic status may predispose an individual to depression (Lorant *et al.*, 2003). Low SES can lead to adversity and life stress, which in turn can serve as a precursor in the translation of SES vulnerability and an individual's risk of developing depression (Dohrenwend, 2000).

According to the Mental Health Foundation, the socio-economic cost of mental health problems outweighs that of crime estimated at 60 billion for England and Wales in 1999/2000 (Brand *et al.*, 2000). The most consistent finding in psychopathology research is the relationship between socioeconomic status (SES) and psychiatric disorders. Research has demonstrated that an inverse linear association including lower SES, correlates with higher depressive symptomatology (Turner and Lloyd, 1999; Lorant *et al.*, 2003).

However, not all individuals with a low SES become depressed. Depression is found even in individuals from a more privileged, higher social class. Therefore, other factors need to be considered, not just social or economic study. For example, Reynolds and Ross (1998) found occupational status was associated with physiological and psychological symptoms of stress (Reynolds and Ross, 1998). It has been reported that job characteristics appear to mediate SES effects on depression (Pappas *et al.*, 1993; Adler *et al.*, 1994; House and Williams, 2000).

1.5.7 STRESS AND DEPRESSION

Chronic stress leads to elevated cortisol levels and reduced serotonin levels along with other neurotransmitter changes, such as dopamine, which has also been linked to depression. When these chemical systems work normally, they regulate many biological processes, such as sleep, appetite, energy, sex drive and emotions. When a stress response causes dysregulations of these hormones after a difficult situation has occurred this could lead to depression or a mood disorder in susceptible people.

As mentioned previously, life stressors and depression are closely interlinked. The involvement of stressful life events has been long implicated and well established in the aetiology of depression (Kessler, 1997; Mazure, 1998; Monroe *et al.*, 1999; Paykel, 2003; Hammen, 2006). Exposure to stressful or adverse life events is suggested to increase the risk of developing depression (Kendler Ks, 2003; Kendler *et al.*, 2006).

However, exposure to stressful life events may predispose an individual to depression, without necessarily increasing the lifetime risk of depression (Kessler, 1997; Richardson *et al.*, 2006). Stressful life events examined in the literature have included: childhood sexual abuse, premature parental loss, lifetime traumas (e.g., natural disasters) and divorce/marital problems.

Both chronic stress (Hammen, 2005; Hammen *et al.*, 2009) and acute stress (Kendler, 1998) have been linked to the risk of predisposition to later development of depression and mood disorders. This correlation, although widely reported, is not necessarily caused by childhood sexual abuse (CSA), confounding influence of disturbed and disadvantageous family backgrounds including impaired parenting, parental marital problems and parent maladjustment (Hawker and Boulton, 2000). Exposure to a disturbed family environment, itself, has been shown to increase the risk of developing depression (Holmes and Robins, 1988; Kessler, 1997; Kendler and Prescott, 2007). Events involving loss of parent, divorce or the threat of separation are associated with depression. However, this relationship is not specific to depression as these events have been shown to precede other illnesses e.g. schizophrenia, anxiety and bulimia (Monroe *et al.*, 1999; Kendler Ks, 2003).

Depressed patients with severe life stress have been found to have greater depressive symptomatology in compared to depressed patients without stress and these effects in symptom severity are primarily due to pre-onset severe events and post-onset events (Monroe *et al.*, 1999).

Researchers have reported that depressed patients typically have approximately 50% incidence of recent severe stress; further supporting the role of stress and the onset of depression (Mazure, 1998). Stressful or adverse life events do not necessarily predict depression or increase the overall risk of developing depression, but may predispose individuals to later developing depression.

1.5.8 PATHOPHYSIOLOGY AND PHARMACOLOGY OF DEPRESSION

Neurochemical Dysfunction

The monoamine Hypothesis opines that depression is the result of monoamine under activity. The monoamine hypothesis proposes that antidepressant drugs have many therapeutic benefits because they increase monoaminergic transmission (Hirschfeld, 2000).

Schildkraut's catecholamine hypothesis of depression specifically suggested the principle involvement of a Noradrenaline (NA) deficiency and other types of dysregulation of receptor functioning (Nestler *et al.*, 2002). The basis for these findings is largely early pharmacological clinical observations (Berton and Nestler, 2006; Pittenger and Duman, 2007). Two structurally unrelated compounds developed for use of non-psychiatric conditions, namely Iproniazid, Imipramine were showed to have potent antidepressant effects in humans

(Nestler, 2002). Iproniazid, a drug studied as an antitubercular agent elevated the mood of tuberculosis patients (Pare and Sandler, 1959).

Further investigation found that Iproniazide inhibits brain monoamine degradation by inhibiting enzyme monoamine oxidase. This resulted in an increase of brain monoamine levels – monoamine oxidase inhibitor (Pare and Sandler, 1959).

Roland Kuhl (1958) conducted the first study using imipramine with depressed patients in 1955. This became the cornerstone in the formulation of the idea of an antidepressant; the results were published in 1958 (Gach, 2008).

Imipramine is a tricyclic compound originally studied as an antipsychotic (Kuhl, 1958) and was found to have potent antidepressant effects which inhibited the reuptake of NA (and to a lesser extent serotonin) into presynaptic neurons (Gach, 2008).

Reserpine compounds have been indicated in the development of depressive states as a potent tranquiliser and the old antihypertensive agent produced a severe depressive state in a subset of patients (clinically indistinguishable from endogenous depression).

Suicide attempts were observed in some individuals and these subjects showed evidence of depleted brain amines (serotonin and catecholamines) (Ferguson, 1955; Stein and Himwich, 1995; Baumeister *et al.*, 2003). Similar behaviours were seen in rodents administered reserpine (Bogdanski *et al.*, 1958; Krishnan and Nestler, 2008).

These observations led two groups of researchers (Bunney Jr and Davis, 1965; Schildkraut, 1965) to argue that NA activity was decreased in depressive disorders and elevated in manic or excited states. Data suggests a biological

heterogeneity of NA in depression as some patients have low NA activity and others have elevated activity (Ressler and Nemeroff, 2000).

Later aetiology of the theories of depression includes the involvement of serotonin (Bogdanski *et al.*, 1958; Meltzer, 1989; Whitaker-Azmitia, 1991; Maes and Meltzer, 1995; Nutt, 2002). Serotonin theories have suggested that there is a decreased production and/ or reuptake in depression (Bogdanski *et al.*, 1958; Raap and Van de Kar, 1999; Hjorth *et al.*, 2000). Most psychotropic drugs are thought to act at least in part on the serotonergic system/mechanisms (Nestler, 2002). Fluoxetine (Prozac) was the first recognised Selective Serotonin Reuptake Inhibitor (SSRI). These groups of drugs were far more selective than the original tricyclic antidepressant (TCA).

The major classes of drugs used to treat depression are: Monoamine Oxides Inhibitors (MAOIs), TCAs and the psychomotor stimulants such as amphetamine (used until the mid 1960s) (Charney *et al.*, 1998; Lieberman, 2003). These agents appear to interact with neurotransmitters in a way that is consistent with the MAO hypothesis of depression. The suggested modes of action of amphetamines (partial agonist, catecholamine reuptake inhibitor, competitive inhibitor of MAO and displacer of presynaptic NA and dopamine) would be expected to increase catecholamines temporally at the receptors sites. Administration of long-term high dose amphetamine produces an eventual depletion of brain NA and dopamine and inhibition of neuronal activity in catecholamine neurons (Kamata and Rebec, 1984).

The largest excitatory neurotransmitter found in the brain is glutamate, which may play an important role in neuropsychiatric disorders. The glutamatergic

system is interconnected with GABAergic and monoaminergic pathways in the limbic system and abnormalities of this system have been found in patients with affective disorders (Kim *et al.*, 1982; Francis *et al.*, 1989; Mauri *et al.*, 1998; Levine *et al.*, 2000; Mitani *et al.*, 2006; Frye *et al.*, 2007).

Compounds targeting the glutamatergic receptors, or glutamate transporters useful for depression have yet to be identified but as this area develops new therapies and associated mechanisms will likely follow.

1.5.9 TREATMENT OF DEPRESSION

Although the concept of antidepressant drugs (AD) to treat depressive illness has existed since the second century (Sartorius, 2001), it was not until the late 1950's that effective antidepressant treatments became widely available (Pinder, 2001). The main agents used in the 19th century were opium and bromides. Most drugs used in the treatment for depression prior to the 1950's were various forms of sedatives (Lieberman, 2003).

In the 1920's opium gave way to barbiturates e.g. paraldehyde was used extensively for the treatment of depression (Lieberman, 2003). From the 1930's stimulants were introduced e.g. amphetamines, in an attempt to treat depression. During this time period shock therapy was introduced and metrazole which was administered to induce an unmodified convulsion. The knowledge that convulsions could be good for improving mental disturbances was not new in medicine at that time, it was first noted by Hippocrates that malaria-induced convulsions in insane patients was able to cure them (Fink, 1984; Abrams, 2002).

Subsequently, electric shocks were then used to induce fits; this procedure is now commonly known as Electroconvulsive Therapy (ECT). The popularity of ECT dramatically decreased in the 1960s and 1970s due to the use of more effective treatments and as a result of a strong anti-ECT movement (Fink, 1984; Abrams, 2002).

ECT has proven to show efficacy in the treatment of depression and is the only somatic therapy from the 1930's that remains in widespread use today in the treatment of mood disorders (Endler, 1988; Fink, 2000).

Below are the lists of new generation antidepressants which are widely prescribed throughout the medical world.

1.5.9.1 Tricyclic Antidepressants (TCA's)

Tricyclic antidepressants block the absorption (reuptake) of the neurotransmitters, serotonin and noradrenaline, making more of these chemicals available at the synapse. This action augments neurotransmission in limbic areas, which elevates mood in depressed patients. These antidepressants also affect other chemical messengers, which can lead to a number of side effects.

Tricyclic Antidepressants (TCAs) are a class of antidepressant drugs first used in the 1950s. They are named after the drugs' molecular structure, which contains 3 rings (Figure 1-3). It was during testing for schizophrenia that TCAs were discovered to be antidepressants, during the late 1950s and 1960s TCAs were marketed for the treatment of depression (Nestler *et al.*, 2002).

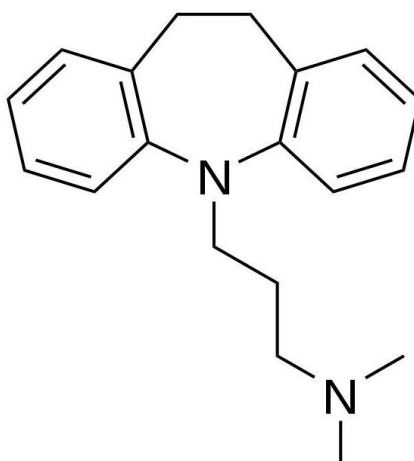


Figure 1-3: Chemical structure of the tricyclic antidepressant (imipramine).

1.5.9.1.1 Mechanism of action

The exact mechanism of action is not fully understood (Dwoskin *et al.*, 2006). They are thought to exert their therapeutic effect by inhibiting the re-uptake pump for monoamine neurotransmitters, serotonin and noradrenaline into the presynaptic neuron, thus enhancing noradrenergic and serotonergic neurotransmission (Einarson *et al.*, 1999). Some TCAs inhibit serotonin reuptake pump (e.g., clomipramine) and others are more selective for NA over 5-HT (e.g., desipramine, maprotiline, nortriptyline, protriptyline) (Einarson *et al.*, 1999). Most TCAs block both serotonin and noradrenaline reuptake to some extent. TCAs as a secondary effect possess an affinity for adrenergic muscarinic and histamine H1 receptors to varying degrees. Although the pharmacological effect occurs immediately, often the patient's symptoms do not alter until 2-4 weeks of persistent administration (Sweetman, 2011).

1.5.9.1.2 Clinical Use

Tricyclic antidepressants are used in numerous applications; mainly indicated for use in clinical depression, neuropathic pain, nocturnal enuresis and Attention Deficit-Hyperactivity Disorder (ADHD). The disadvantages of TCAs include unwanted pharmacological actions including a blockade of muscarinic cholinergic receptors, histamine 1 receptors, alpha1 adrenergic receptors and voltage sensitive sodium channels. Blockade of H1 receptors may cause sedation and weight gain. Blockade of M1 muscarinic cholinergic receptors cause the 'anticholinergic side effects'; dry mouth, blurred vision, urinary retention and constipation. These side effects necessitate starting with a low dose and increasing slowly (Furukawa *et al.*, 2002). Blockade of alpha-1-adrenergic receptors causes orthostatic hypotension and dizziness. TCA overdose is a significant cause of fatal drug poisoning. The severe morbidity and mortality associated with all TCAs except lofepramine, is well documented due to their ability to weakly block sodium channels in the heart and brain resulting in cardiovascular and neurotoxicity (Buckley *et al.*, 1994; Cohen *et al.*, 2000).

This toxicity and the perceived poor tolerability of these drugs in general have led to a decline in their use in the UK over the last decade (NICE, 2009).

1.5.9.2 Monoamine Oxidase Inhibitors (MAO's)

This is the group of the older class of antidepressants with limited, but selective use for the treatment of depression (Livingston and Livingston, 1996; Berton and Nestler, 2006). Monoamine Oxidase Inhibitors (MAOIs) are a class of powerful antidepressant drugs prescribed for the treatment of depression. The

first antidepressant drug synthesised was an irreversible MAOI and drugs in this class have been available in the UK for nearly 50 years. Although recognised as powerful antidepressants, the MAOIs are also highly effective therapeutic agents for certain anxiety disorders, such as panic disorder and social phobia, and in smoking cessation.

1.5.9.2.1 Mechanism of action

MAOIs inhibit the activity of monoamine oxidase, an enzyme present in various parts of the body, thus preventing the breakdown of monoamine neurotransmitters and thereby increasing their availability. There are two isoforms of monoamine oxidase, MAO-A and MAO-B. MAO-A preferentially deaminates serotonin, melatonin, adrenaline and noradrenaline (Bortolato *et al.*, 2008). MAO-B preferentially deaminates phenylethylamine and trace amines. Both types equally deaminate dopamine. Both forms are inhibited by the original MAOIs, which are therefore non-selective. Original MAOIs are all irreversible enzyme inhibitors meaning they bind to MAO covalently and irreversibly and destroy its function indefinitely (Benedetti and Dostert, 1985). Enzyme activity returns only when a new enzyme has been synthesised, which can take approximately 2 weeks. Newer MAOIs, are reversible, notably moclobemide, they have the ability to detach from the enzyme to facilitate usual catabolism of the substrate (Robinson, 2002).

1.5.9.2.2 Clinical Use

MAOIs tend to be underutilised in clinical practice now, even in hospitalised patients. This is due to the potential lethal dietary and drug interactions and the availability of safer antidepressants. All MAOIs have the potential to induce hypertensive crisis if foods containing tyramine (which is also metabolised by monoamine oxidase) are ingested (Merriman, 1999) or drugs that increase monoamine neurotransmission are co-prescribed (Livingston and Livingstone, 1996).

In the past, MAOIs were used for patients resistant to TCAs, but the newer reversible MAOIs (moclobemide) provide a safer alternative and are now occasionally used as first-line therapy, although these substances are not as effective as the original MAOIs (Da Prada *et al.*, 1989). The three of the original MAOIs that are still available in the UK for clinical use today are phenelzine, tranylcypromine and isocarboxazid. MAOIs are still cited as being the most effective antidepressants for the treatment of atypical depression (McGrath *et al.*, 1994; Nolen *et al.*, 1994).

1.5.9.3 Selective Serotonin Reuptake Inhibitors (SSRI's)

Selective serotonin reuptake inhibitors (SSRIs) were introduced in the late 1980s and they have dramatically revolutionised clinical psychopharmacology. It is predicted that 6 prescriptions per second, around the clock, around the year, are written for these agents (Anderson, 2001; Ban, 2001).

1.5.9.3.1 Mechanism of Action

The Selective Serotonin Reuptake Inhibitors (SSRIs) inhibit the reuptake of serotonin; each SSRI shares the common property of serotonin transporter (SERT) inhibition. SSRIs bind at the SERT and prevent 5HT from being taken up both at the axon and dendrites, leaving more neurotransmitter in the synapse and providing an antidepressant effect. While SSRIs have in common their high affinity for 5-HT reuptake inhibition, each has a secondary pharmacological action, which includes NRI (noradrenaline reuptake inhibition), DRI (dopamine reuptake inhibition), 5-hydroxytryptamine 2C receptor (5-HT_{2C}) antagonism, muscarinic/cholinergic antagonism, sigma-1 receptor actions and inhibition of Nitric Oxide Synthase (NOS) and of CYP450 2D6, 3A4 and 1A2 (Iversen, 2006).

1.5.9.3.2 Clinical Use

Selective serotonin reuptake inhibitors are used in the treatment of depression, anxiety disorders, obsessive-compulsive disorder and bulimia nervosa, via 5-HT_{1A} antagonism (Geddes and Butler, 2002; Cipriani *et al.*, 2005; Cipriani *et al.*, 2009). This class of antidepressants typically have fewer adverse events and side effects than TCAs and MAOIs. SSRIs are less cardiotoxic than TCAs, have fewer anticholinergic side effects (e.g. sedation) and low toxicity in overdose (Gunnell *et al.*, 2005; Möller *et al.*, 2008). Dosage titration is not routinely required so subtherapeutic doses are less likely to be prescribed. These advantages have led to their widespread use as better-tolerated first-line antidepressants (Bosker *et al.*, 2004).

1.6 ANIMAL MODELS

1.6.1 GENERAL CONSIDERATIONS IN MODELLING DEPRESSION

A study by Duman (2010) reports that a basic understanding of the underlying disease processes in depression is currently lacking and therefore it is not possible to recreate the disease in animal models with precision (Duman, 2010).

Animal models attempt to produce quantifiable correlates of human depression symptoms in experimental animals. It has been reported that it is not possible to assess the most classical symptoms of depression in animals because most of these symptoms are subjective feelings. Therefore, we strive to model the symptoms of depression that can be easily translated to behaviours that are measurable in animals. Present animal models for depression differ considerably in the extent to which they produce features that resemble depressive-like behaviours and that mimic human depression. The examples of measures that can be evaluated in rodent behavioural models include; social interaction, motor responses to stress and reward-related responding. The rationale for these is that they reflect levels of helplessness or despair, social withdrawal and anhedonia, all of which measures are characteristic of human depression. Psychomotor, appetite and sleep alterations, are not uniformly useful for investigating the neurobiological mechanisms of depression as these behaviours are sometimes elevated, or diminished in depression (Duman, 2010).

The ability of animal models to produce animal aspects of depression illness seen in humans is generally evaluated in their reliability or reproducibility (e.g.

face validity), their ability to precisely predict outcome in humans (e.g. predictive validity) and the incidence to which they model the disease process or its etiology in humans (e.g. construct validity) (Willner, 1984; Willner, 1997; McKinney, 2001). Clinically, predictive validity is the ability of animal models to accurately detect treatments that are useful for depression.

Pharmacologically, the utility of many of the models is based on their predictive validity. This consideration has become increasingly important as new emerging evidence suggest that neural plasticity is involved in antidepressant drugs effectiveness (Pittenger and Duman, 2007).

This emphasizes the importance of using animal models with features that result from processes believed to be relevant to human depression (Duman, 2010).

Because of the lack of understanding for the pathophysiological basis for depression, models are used from poor construct validity. Although different animal models attempt to produce specific behavioural or physiological features of depression, the features in the animal models likely come about through processes that are very different from those operative in human depression. Therefore, results obtained from using animal models need to be carefully interpreted for their relevance. This is because there is a limitation in using animal models in that they are used to detect mechanisms of current antidepressant drugs in animals and may lack the ability to detect potentially novel mechanisms that actually underpin human depression (Duman, 2010).

1.6.2 ANIMAL MODELS OF DEPRESSION

One of the key environmental risk factors thought to evoke depression is the exposure to stress (Keller *et al.*, 2007; Kendler *et al.*, 1999; Kessler, 1997). Thus, early-life adverse experience could lead to psychopathology in humans. Studies carried out on humans show that exposure to stress or early adversity in life increases the risk for depression and that stress exposure may promote depression in those with genetic risk factors (Agid *et al.*, 1999, 2000; Caspi *et al.*, 2003; Kaufman *et al.*, 2006; Weiss *et al.*, 1999). Early Life Stress exposure (ELS) model has been developed to investigate determinants of experience-dependent susceptibility for developing depressive illness (Duman, 2010). Animal models to study neurobehavioural function permit researchers precise control in studies as detailed below (Table 1.3) (Hitzemann, 2000).

Table 1.3: Reasons for using animals in the study neurobehavioural function.
Adapted from (Katz, 1981).

1	Control of and manipulation of genetic history.
2	Short life span allows longitudinal study, within and across generations.
3	Direct experimental manipulations of examination of the brain possible.
4	Full control of life history.
5	Cost effective and time effectiveness.

Many studies show that exposure to stress, can reliably elicit many core components of depression (Duman, 2010).

Physiologically, it appears that different types of stressors in animals activate different neural circuits (Duman, 2010). For example, there is a differential involvement of limbic pathways thought to underpin the processing of stressors that vary in their systemic (i.e. immune, metabolic, cardiovascular and metabolic which promote the adverse actions) versus cognitive/psychological nature (i.e. stressors which lead to excessive release of CORT) (Herman and Cullinan,

1997; Anisman and Matheson, 2005). Importantly, the degree of control an animal has when it is stressed has been demonstrated to be the key factor for the neurobehavioural consequences of stress exposure. For example, behavioural impairments and increased brain amine utilisation can be seen after exposure to uncontrollable stress, but are not apparent when an animal is able to control/predict the stress exposure (Anisman and Matheson, 2005). Greater unpredictability can reduce the probability of adaptive processes occurring upon repeated stress exposure and promotes the appearance of stress related effects (Willner *et al.*, 1987; Anisman and Matheson, 2005).

According to Van Der Staay *et al.* (2009) validation of a model is a scientific method to improve the confidence in that model, i.e. to evaluate its plausibility and consistency (Van Der Staay *et al.*, 2009). Even though numerous definitions on validity have come into existence, the most notable one is defined as “ the agreement between a test score or measure and the quality it is believed to measure “ (Silva, 1993). Validity should not be interpreted as the “truth” of a model but an interpretation of the data arising from the model. Validity in this sense is a major criterion for evaluating animal models in practical terms (Holmes, 2003b). According to Silva (1993), no animal model can be valid in all situations, for all purposes. Validity is restricted to a specific use of the model and consequently, must always be open for discussion and re-evaluation (Silva, 1993).

There is no general consensus about how to rank the different categories of validity in the model evaluation process. We hold that the validation process should consider the internal validity which includes reliability and replicability,

face validity, predictive validity, construct validity and external validity of a model. The concepts of internal validity, face, predictive and construct validity have been elucidated in a number of publications e.g. (Willner, 1984; Willner, 1986; van der Staay, 2006).

1.6.3 VALIDITY OF ANIMAL MODELS OVERVIEW

The following sections will develop and introduce basic concepts of animal models and move onto a detailed examination of animal models for depression to fully illustrate these concepts.

1.6.3.1 Reliability and replicability, internal validity

Reliability is a measure of the assessment instrument whereas replicability (or reproducibility) is a quality of the results obtained using a particular animal model (Van Der Staay *et al.*, 2009).

Reliability explains the consistency of the assessment or testing device, i.e. it explains the extent to which a measurement instrument yields consistent results each time the measurements are performed under the same experimental conditions (Van Der Staay *et al.*, 2009).

Internal validity refers to the quality of the experimental appraisal of the animal model, for example how well the research was designed, how variables are measured, what variables were included, performance of a study, how strictly variables were controlled and about the confidence levels of how one can be sure that the changes observed in the dependent variable(s) are induced by

experimentally manipulating the independent variable(s) and not by factors that might also affect the independent variable and may offer alternative explanations of the results obtained (Guala, 2003).

Internal validity is a measure of a specific lab's ability to obtain that result. It is most obviously demonstrated in studies from a given laboratory.

1.6.3.2 Face validity

Face validity is the extent of descriptive similarity between the behavioural disorders seen in an animal model and in the human condition that the model seeks to evaluate. The similarity of symptoms may be the beginning point of identifying a possible animal model of neurobehavioural dysfunction (Geyer and Moghaddam, 2002). Although face validity has been suggested to constitute a major or even the most important gauge for model appraisal by (Holmes, 2003a), strong emphasis on face validity has been criticized (Sarter and Bruno, 2003).

1.6.3.3 Predictive validity

Predictive validity is the measure of how well a test predicts the future performance of the test. It is also known as criterion validity (Van Der Staay *et al.*, 2009). An animal model with high predictive validity predicts behaviour in the situation it is thought to model. It allows extrapolation of the effect of a particular experimental manipulation from one species to another and from the laboratory to the 'Real World' (Epstein *et al.*, 2006).

In psychopharmacology a narrower concept of predictive validity is used (e.g. (Sarter *et al.*, 1992; Bourin *et al.*, 2001; Borsini *et al.*, 2002; Cryan and Slattery, 2007; Whiteside *et al.*, 2008) where it is considered to be of particular importance in drug development programs (Swerdlow and Sutherland, 2005). In this context, predictive validity refers to an animal model's utility to correctly identify the efficacy of known or putative therapeutic agents in the treatment of a particular disorders (Wright, 2002).

1.6.3.4 Construct validity

Construct validity is the relationship between the measurements (dependent variables) and of the manipulations (i.e. independent variables) with the theoretical hypotheses being tested (Lubow, 2005). According to Sarter and Bruno (2003), construct validity is the most important gauge for animal models because it addresses the soundness of the theory underlying the model and it identifies the framework for translating data generated by the model (Sarter *et al.*, 1992; Sarter and Bruno, 2003).

Epstein (2006) has reported that construct validity points to the degree of similarity between the mechanisms underlying the behavioural disorder being modelled and the mechanisms underlying specific behaviours observed in this model (Epstein *et al.*, 2006). Construct validity thus is a theory which is driven by experimental substantiation of pathophysiological, behavioural and/or neuronal components of the model (Sarter and Bruno, 2003), i.e. it reflects the degree of fitting the theoretical rationale underlying the model with the true

nature of the syndrome/symptoms that the animal model mimics (Holmes, 2003b).

Numerous studies have defined the theory of construct validity and perhaps the most noted is one by Kaplan and Silva which defines a framework of theoretically relevant relations in the model (Silva, 1993; Kaplan and Saccuzzo, 2008). This reflects the soundness of the theoretical rationale of the disorder (Wright, 2002).

Anxiety and depression are both mood disorders and often co-morbid. An effective animal model must clearly delineate between each disorder.

1.6.4 TYPES OF ANIMAL MODELS OF DEPRESSION

1.6.4.1 Learned helplessness

When animals are exposed to inescapable stress (e.g. electrical footshock) for more than one session, this procedure may result in Learned Helplessness (LH). Subsequent to inescapable stress, the animals are tested for their ability to engage in an active avoidance task. In these models, animals are prevented from escaping the electrical footshock in a shuttle box chamber. Animals previously exposed to inescapable stress (e.g. electrical footshock) show decreased abilities to escape when afforded the opportunity to do so. Antidepressant drugs e.g. MAOIs, TCAs, SSRIs and ECT usually restores the ability of the animals to escape (Sherman *et al.*, 1982; Martin *et al.*, 1990).

Willner (1984) found this model has a good predictive validity for the efficacy of antidepressant drugs (Willner, 1984). The LH model has been used to show the importance of the controllability of the inescapable stressor as a key

psychological component in inducing the behavioural problems (Anisman and Matheson, 2005).

Maier (1984) concluded in his review that there is a great similarity between human depression symptoms (e.g. weight loss, decreased motivation, increases in stress hormones and decreases in motor activity) and the behaviours associated with the learned helplessness model in rodents.

(Maier, 1984).

1.6.4.2 Porsolt Test or Forced swim test

This study was first described by Porsolt et al. in 1977 and involves placing a rat or mouse in a chamber full of water such that the animal cannot touch the chamber bottom with its hind paws (Porsolt *et al.*, 1977a; Porsolt *et al.*, 1977b; Porsolt *et al.*, 1978).

A normal animal shows an initial struggling reaction and tries to escape by jumping, climbing until eventually it adopts an “immobile” posture, where it maintains its snout above the water line to breathe. It is a 2 day test with the first day designated the “exposure” day and on the 2nd day, termed “test” day, where total immobility is measured.

The exposure day primes the animal to produce immobility on the test day. Greater immobility is deemed to be depressive-like behaviour. It therefore belongs to the spectrum of tests that employ the concept of learned helplessness as a feature of depression. It is commonly used to screen novel antidepressants, since testing with known antidepressants produce less immobility on the test day (Porsolt *et al.*, 1978).

The monoamine oxidase inhibitors, tricyclic and atypical antidepressants (e.g. bupropion, nefazodone, trazodone, mirtazapine) have all been shown to decrease the duration of immobility in rodents in a dose-dependent manner (Porsolt *et al.*, 1977a; Porsolt *et al.*, 1977b; Borsini and Meli, 1988).

Stimulant drugs (e.g., amphetamine) may cause false positive results in the Porsolt test, because this drug can increase locomotor activity and therefore decrease immobility (Katz *et al.*, 1981). In addition, the Porsolt test does not uniformly distinguish between acute and chronic antidepressant effects. Several studies have shown that the Porsolt test is sensitive to genetic variation as indicated by differences in strain efficacy of drug in rats and mice (Porsolt *et al.*, 1978; López-Rubalcava and Lucki, 2000).

1.6.4.3 Tail suspension test

The Tail Suspension Test (TST) is conceptually similar to the Porsolt test in that it suspends a mouse by its tail, while measuring the extent to which immobility versus active movement (Steru *et al.*, 1985). In a stress situation, the TST similar to the Porsolt test is based on the adoption of a passive response.

The TST has good predictive validity; and several studies have shown that acute antidepressant treatment given prior to the test decreases immobility time in the TST (Steru *et al.*, 1985; Perrault *et al.*, 1992; Cryan *et al.*, 2005). Although, the TST is similar to the Porsolt test, both tests do not show congruous sensitivities to pharmacologic agents or to any differences in strain, suggesting that responding in the TST and the Porsolt test may be determined by non-congruous substrates (Bai *et al.*, 2001).

There are limitations with the TST in comparison to the Porsolt test. The major drawback of the TST is that it is only used in mice and not in rats due to size and weight whereas the Porsolt is suitable for both types of rodents.

The TST and Porsolt test have similar limitations, including sometimes showing false positive responses to psycho-stimulants (e.g., amphetamine). Nevertheless, the high reliability of the TST and Porsolt test has contributed to their sustained use and they are both considered useful for investigating differences between strains to stress and in assessing depression-like behaviour (Duman, 2010).

None of the above mentioned models, i.e. LH, the Porsolt test, and TST reproduce the pathophysiology of depression, but they are useful in that they are sensitive to therapeutic agents in a manner predictive of their effects in humans. The Porsolt test and TST have been used extensively for this purpose, but the selectivity of these tests for monoamine-based mechanisms may limit their ability to detect novel mechanisms (Willner, 1991; Thiebot *et al.*, 1992; Weiss, 1995; Lucki, 1997).

1.6.4.4 Chronic unpredictable mild stress

Learned helplessness, elicited by the Porsolt test, and tail suspension test procedures depend on short term stress exposure, but the Chronic, Unpredictable mild Stress (CUS) test was developed to study neural changes which result from stress of a more chronic nature (e.g., wet sawdust, cage tilting, sounds of a predator, water in the bottom of an empty cage, being placed

in an empty cage, during the dark phase lights will be on for a short duration, and moving cages).

This procedure lasted for 28 days before behavioural tests (e.g. open field, elevated plus maze, Porsolt test and a tail suspension test) (Mineur *et al.*, 2003). The advantage of over the aforementioned stress procedures is the intuitive belief that frequent different stress stimuli, each varying in physiological effects is a more natural reflection of major life stress experienced by humans. Thus it is the aggregate of their effects summated over long time periods that cause depressive-like behavioural responding; at first these responses are mild, but eventually become sensitised and elicit a more robust depression.

One of the first studies carried out on the CUS model was by Katz and colleagues and then developed by other researchers providing the basis for most of the currently used tests (Katz, 1981; Katz *et al.*, 1981; Willner *et al.*, 1987; Willner, 1997).

1.6.4.5 Sucrose preference

Anhedonia is defined as a loss of interest or inability to experience pleasure, even with activities that were previously highly rewarding. Measuring alterations in anhedonia represents another technique to measure depression-like behaviour in rodents (Willner, 1984; Willner, 1991).

Sucrose preference test is used to examine the animal's interest in their activities e.g. sexual, social, motivation, and food (Willner *et al.*, 1987; Deussing, 2006). The benefit of this test procedure is that the behaviour to drink is not elicited via an aversive event, but rather elicited by the degree to which the animal is motivated by pleasant taste of sucrose (Willner *et al.*, 1987).

Sucrose preference testing is carried out in the animal's home cage. For the task, animals are presented with a choice to drink plain water or 2-4% sucrose solution in water (Willner *et al.*, 1987; Papp *et al.*, 1991).

Animals much prefer to drink more sucrose water than plain water, and thus any procedure applied to the animals prior history such as chronic (presumably leading to a depression-like behaviour state), would be expected to reduce the innate preference of the animal for the sucrose solution. Diminished sucrose intake can be inferred to indicate that the animal no longer finds it as pleasurable (Brenes Sáenz *et al.*, 2006; Brenes and Fornaguera, 2008). Human depressed patients exhibit a similar diminution in interest of pleasurable activities, and the sucrose-preference test models this precise symptom of depression (Willner *et al.*, 1987; Papp *et al.*, 1991; Willner, 1991; Willner, 1997). A number of studies have shown that chronic antidepressant treatment can reduce anhedonia seen in CMS rats without producing general changes in fluid intake, palatability, or learning capacity (Willner *et al.*, 1992; Treit and Menard, 1998) .

One caveat is of course that sucrose supplies a calorie load to the animal, and this may produce modifications in the amount of nutritionally vital dietary components such as vitamins, minerals, lipids and proteins (Wooley *et al.*, 1972; D'Aquila *et al.*, 1997).

Artificial sweetness' such as saccharine can be used to control for these problems, but may have deleterious consequences of their own (Kurre Nielsen *et al.*, 2000)

1.6.4.6 Early-life stress

Experimental paradigms have been developed in an effort to model Early Life Stress (ELS) and are used as models in which to investigate determinants of experience-dependent susceptibility to depressive illness (Agid *et al.*, 1999; Weiss *et al.*, 1999; Agid *et al.*, 2000; Caspi *et al.*, 2003; Kaufman *et al.*, 2006). Stress exposure during critical periods of development can cause stable phenotypic changes, which are induced by the ELS models.

ELS produce changes that are particularly replicable and involve alterations in neural systems that control or respond to stress such as the HPA axis; and include neurochemical, endocrine and behavioural changes. Changes in HPA axis which regulate stress systems after ELS could be relevant to consequences in humans after early life stress and may lead to suggest mechanisms that predispose to depressive illness (Heim *et al.*, 2004; Pryce *et al.*, 2005).

1.6.4.6.1 Maternal separation

Several studies show that parental care is evidently an important modifier of stress exposure during development in humans and maternal deprivation tests are useful in developmental animal models for predisposition of affective disorders/depression (Heim and Nemeroff, 2001; Kendler *et al.*, 2002; Newport *et al.*, 2002a; Holmes *et al.*, 2005). It has been suggested that maternal behaviour has a role in programming emotion-related behaviour in offspring caused by translating the stress level in the environment into the offspring via parental behaviour (Zhang *et al.*, 2006).

Several maternal deprivation models exist which involve repeated periods of separation of preweaning rats from the dams. Preweaning rats are exposed to daily episodes of 3–6h separation in the first 2 postnatal weeks and the separation can include removing the entire litter from the dam, or removal of individual pups from their littermates and the dam (Rüedi-Bettschen *et al.*, 2005; Vazquez *et al.*, 2005).

Previously separated animals are then allowed to develop into adulthood, under normal conditions where phenotypic characteristics can be evaluated (Lovic *et al.*, 2001).

Several studies have indicated that rats separated during early life show behavioural abnormalities, for example, reduced social motivation, appetite and sleep problems, reduced pleasure, decreased motor activity, increased anxiety and fear responses and endocrine neurochemical changes in stress-relevant systems as adults (Levine, 1957; Plotsky and Meaney, 1993; Ladd *et al.*, 2000; Mintz *et al.*, 2005; Rüedi-Bettschen *et al.*, 2005; Rüedi-Bettschen *et al.*, 2006).

The behavioural changes that accompany Maternal Separation (MS) show similarities with symptoms of depression and the neuroendocrine alterations are also consistent with depression (Pryce *et al.*, 2001; Heim *et al.*, 2004; Pryce *et al.*, 2005). Chronic antidepressant drug treatment counteracts some effects of maternal separation (Leventopoulos *et al.*, 2009), but generally predictive validity needs further studies (Duman, 2010).

The brain is very susceptible to experience-dependent alterations during the critical period of postnatal development. Newport *et al.* (2002) reported that maternal separation test in rodents target this period (Newport *et al.*, 2002b).

Parental behaviour contains direct or indirect behaviour at the offspring; direct behaviour (arched back nursing, licking grooming and retrieval), or nest building and indirect behaviour which includes protecting their offspring from any threat conditions that might be harmful (Bornstein, 2002).

There are positive and negative approaches to conceptualizing parenting behaviour. Positive behaviour is associated with the growth of litter's proficiency in life areas and behaviours control. Adverse or negative behaviour in comparison suggests a parent as a factor in the development of emotional-behavioural problems, in particular anti-social behaviour and violence (Menendez-Patterson *et al.*, 1982). So, these models have face validity for disrupted parenting behaviour in humans that can develop from a number of situations including father or mother depression and also likely to occur during critical periods of the individual's development (Newport *et al.*, 2002).

A variation of the MS procedure is a related paradigm that uses the quantification of levels of maternal care as it occurs naturally rather than via experimental manipulations (Cannon, 1939; Liu *et al.*, 1997a; Francis *et al.*, 1999b).

Champagne *et al.* (2003) found that the licking/grooming of pups and arched back nursing are important features of maternal behaviour in female rats (Champagne *et al.*, 2003). These maternal behaviours, which are naturally occurring variations are quantified and low levels of these variations are considered to represent a stress condition for the offspring (Duman, 2010). Levels of maternal care have been shown to associate with levels of stress-

reactivity (HPA activity and anxiety-related phenotype) in the adult offspring (Liu *et al.*, 1997).

1.6.4.6.2 Prenatal stress

In different laboratories, prenatal stress paradigms have been used as a model of depression. It has been reported that maternal stress of different types, for example, restraint during gestation or noise exposure results in changes in the offspring, including changed HPA axis activity, increased symptoms of depression in animal models of depression and increased anxiety (Alonso *et al.*, 1991a; Weinstock *et al.*, 1992; McCormick *et al.*, 1995; Secoli and Teixeira, 1998; Maccari *et al.*, 2003b; Morley-Fletcher *et al.*, 2003; Morilak and Frazer, 2004; Smith *et al.*, 2004).

Prenatal stress tests have construct and face validity, but the depression-related changes and anxiety that are induced in the dams complicate the interpretation as to the relative contributions of pregnancy versus the effect of postnatal care (Duman, 2010).

Studies reported that chronic antidepressant treatment reverses the behavioural changes in Porsolt model of depression, anxiety and HPA axis alteration, which result from prenatal stress (Morley-Fletcher *et al.*, 2004; Poltyrev and Weinstock, 2004). Weinstock, (1997) reported that changes in the rodents' and monkeys' HPA axis are similar to those caused by prenatal stress in humans which lead to behavioural abnormalities e.g. ADHD (Clements, 1991), depression, criminal, unsociable and inconsiderate behaviours (Meijer, 1985) and schizophrenic episodes (Huttunen and Niskanen, 1978).

1.6.5 ANIMAL MODELS OF ANXIETY

Anxiety is adaptive when mild which helps the body to deal and cope with it, but may be crippling and disturbing when it is extreme (American Psychiatric, 2000). Clinically, anxiety appears in several recognisable forms. Differences are made between patients who experience psychological feelings of panic, continuous nervousness, tension and worry; often these are accompanied by autonomic hyperactivity in the absence of external stressors (Schlaepfer and Nemeroff, 2012).

Examples of anxiety include panic attacks which are accompanied by subjective feelings of terror, fear of dying and apprehension. Dyspnoea, faintness, sweating and trembling are somatic symptoms which occur co-morbidly in multiple physiological systems of these individuals (Schlaepfer and Nemeroff, 2012).

Most diagnostic constructions are made for patients presented with anxiety due to phobic disorders, PTSD and obsessive-compulsive disorder (OCD), as reflected in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV: American Psychiatric Association, 1994).

In anxiety research, animals are used widely to screen new anti-anxiety drugs which have anxiolytic effects to treat anxiety in humans (McMillan, 2008). For example, anxiolytic compounds like benzodiazepine drugs have shown to decrease anxiety-like behaviour in rats (Treit, 1991; Treit *et al.*, 1993). Furthermore, betacarbolines are used as anxiogenic agents to induce anxiety in humans; this compound is found to produce anxiety-like behaviour in animals (Ninan *et al.*, 1982; Dorow *et al.*, 1983).

Elevated Plus Maze

The Elevated Plus Maze (EPM) is perhaps the most widely used animal anxiety model in use today especially for rats and mice (Walf and Frye, 2007). It comprises of two open arms (i.e. no enclosing walls) and two closed arms (with barriers on each side). Rats are curious animals and naturally explore the environment (White, 1959; Martin and Bateson, 1993; Cavigelli, 2005). However, according to (Rodgers and Cole, 1993; Padovan and Guimarães, 2000; Adamec *et al.*, 2004), stressors often increase anxiety-like behaviour.

The amount to which the rat is willing to explore the open arms is a measure of the rat's intrinsic anxiety because the animal wishes to explore/escape, but will be vulnerable when in the open (Pellow *et al.*, 1985; Pellow and File, 1986; Adamec *et al.*, 2004). Moreover, the plus maze is a very simple test and provides a high degree of utility in measuring emotional reactivity to experimental drugs.

1.7 PROGRAMMING

1.7.1 OVERVIEW

Mammalian foetuses are exposed to continual chemical signals from conception until birth, transported via the maternal blood supply. Among these signals, a pronounced role is played by the hormones associated with the stress response. Studies of human and nonhumans show that maternal stress during pregnancy exerts pervasive, long-lasting effects on the development of the foetal nervous system and may eventually affect the offspring's physiology and

behaviour (Weinstock, 2008; Baker *et al.*, 2009; Lupien *et al.*, 2009; Conrad, 2011; Del Giudice, 2012; DiPietro, 2012).

The classical approach in psychology and medicine to maternal stress has been to treat it as a disruptive influence on foetal development and a net risk factor for future pathology (Van den Bergh *et al.*, 2005; Weinstock, 2005; Mennes *et al.*, 2006).

Conversely, the last decade has witnessed the emanation and consolidation of another model, based on evolutionary biology, in which foetal programming by maternal stress is seen as an evolved adaptive process (Matthews, 2002; Kaiser and Sachser, 2005; Kapoor *et al.*, 2006; Talge *et al.*, 2007; Kaiser and Sachser, 2009; Glover *et al.*, 2010; Pluess and Belsky, 2011; Sandman *et al.*, 2011).

In humans, maternal stress has been linked with poor coping behaviour under adversity, aggressive and antisocial behaviour, ADHD and depression in children and adults (Weinstock, 2008).

Studies in a wide range of species show the major hormonal systems which mediate stress responses (including the autonomic nervous system (ANS) and HPA axis) can be altered during early life (Plotsky and Meaney, 1993; Liu *et al.*, 2000a; Huot *et al.*, 2002; Read *et al.*, 2005; McEwen, 2008). Most of the studies have been done with respect to the HPA axis, which can be altered or 'programmed' prenatally by nutrient restriction, exposure to synthetic glucocorticoids or maternal adversity and postnatally by neonatal handling, maternal deprivation or even infection to a certain extent (Matthews, 2002).

Evidence from epidemiological, clinical and experimental studies has clearly shown a close link between adverse effects in utero environment and the increased risk of psychological, neurological and psychiatric disorders in later life (Li *et al.*, 2012).

Epigenetic phenomenon play a major role in this process such that environmental signals can be transmitted from the mother to the foetus, impacting upon specific tissues in their sensitive developmental stages; modulating normal development trajectory, remodelling their structure and function and reprogramming the resiliency or susceptibility for diseases during postnatal life (Harris and Seckl, 2011). Such programming processes may be determined by factors which include gestational age, duration, mode of exposure and the nature of the stressor (Harris and Seckl, 2011).

Studies have indicated that foetal stresses such as hypoxia, malnutrition, substances exposure (nicotine, alcohol and cocaine) and excess glucocorticoids (endogenous or exogenous), have a long lasting impact on the developing brain; altering the brain's ontogeny, organisation, structure and function; remodelling the brain's developmental trajectory and reprogramming its vulnerability or resiliency to some neurobehavioural, neuropsychological and neuropsychiatric disorders in later (Seckl and Meaney, 2004c; Harris and Seckl, 2011).

Considering the above factors it becomes evident that early-life environmental factors for the mother effect prenatal development and may cause permanent structural and functional changes. Conceptually, we now refer to this as early-life programming (Meaney *et al.*, 1996a).

The major programming factors responsible for these development changes include nutrients and hormones (Meaney *et al.*, 1996a; Seckl, 1998). For example, highly lipophilic hormones like sex steroids can easily cross the placenta and the blood brain barriers. These are powerful mediators of early-life organizational effects (Seckl, 1998; Seckl *et al.*, 2000).

Prenatal exposure to different types of steroid hormones can be followed to see the effects of programming as in the case of early exposure to glucocorticoids and early-onset hypertension (Seckl, 2004).

Animal models of maternal stress feature a wide range of species, e.g. mice, rats, guinea pigs and nonhuman primates. These have demonstrated that prenatal stress increases offspring HPA axis sensitivity, anxiety and depressive-like behaviours, as well as cognitive deficits (Kapoor and Matthews, 2005; Mueller and Bale, 2007; Darnaudéry and Maccari, 2008). Similarly in epidemiological findings, animal models of maternal stress found a temporal specificity of stressor exposure and foetal sex were predictive factors (Bale *et al.*, 2010).

Studies showed that early life experiences enhanced prefrontal-dependant response, increased exploration behaviour, decreased levels of cortisol and reduced anxiety in primates (Parker *et al.*, 2005; Katz *et al.*, 2009).

A study by King *et al.* (2009) revealed that acute prenatal maternal stress, following exposure to a life-threatening ice storm during the gestational period for fingerprint development (the second trimester) resulted in greater dermatoglyphic asymmetry in the children. This was most pronounced in those with greater maternal distress (King *et al.*, 2009).

1.7.2 DEFINITIONS OF PROGRAMMING

There are different concepts which arise with regards to programming. Several studies have reported that physiological programming of early life experiences explains the links between prenatal environment events, changed development and growth of the foetus and later disease states e.g. hypertension, type 2 diabetes, autism and depression (Csaba, 1986; Barker *et al.*, 1993; Edwards *et al.*, 1993; Seckl, 1998; Seckl and Meaney, 2004a).

Various definitions are offered to explain programming, but the most notable perhaps comes from Fink (2000). He defines it as the process through which a stimulus or insult, when applied during a sensitive or critical period of development, results in a long-term or permanent effect on the structure or function of the organism (Fink, 2000).

Subsequently, the concept of early life programming came into existence and it defines the action of an external factor during a sensitive developmental period, or critical period, this affects the development and organization of specific tissues that produces effects persisting throughout life (Fink, 2000).

At different occasions and time points, cells and tissues are programmable and vulnerable to the effects of environmental challenges (Seckl and Meaney, 2004a).

1.7.3 MECHANISMS OF PROGRAMMING

It is clearly understood that higher HPA axis activity confers enhanced response to stress and challenge, underpinning some neurobehavioural and psychiatric abnormalities (Seckl and Meaney, 2004c; Cottrell and Seckl, 2009; Harris and Seckl, 2011). The HPA axis and its key limbic regulator, the hippocampus

(Jacobson and Sapolsky, 1991), are particularly sensitive to hormones especially the glucocorticoids and their actions (Gould *et al.*, 1991; Welberg *et al.*, 2001; Gould *et al.*, 2004; Seckl and Meaney, 2004a).

As the function of the nervous and endocrine systems is closely integrated with behavioural responses, Weinstock (2001) reported that there is evidence that behaviour can be programmed during development (Weinstock, 2001). Gluckman and Hanson (2004) suggested that this process is helpful from an evolutionary perspective allowing organisms to adapt during a single generation (Gluckman and Hanson, 2004).

Studies have reported that prenatal glucocorticoid exposure permanently increases basal plasma corticosterone levels in adult rats (Levitt *et al.*, 1996; Welberg *et al.*, 2001). Thus, perinatal glucocorticoid exposure increase blood pressure and blood glucose levels in adulthood (Seckl, 2004).

For example studies have shown that administration of dexamethasone (a synthetic glucocorticoid used during obstetric practice) to pregnant rats leads to reduced birth weight, which reverses by day 21 (Seckl, 2001; Seckl, 2004; Seckl and Meaney, 2004b).

However, both male and female adult offspring of dexamethasone-treated pregnancies show elevated blood pressures (Benediktsson *et al.*, 1993). Similar adult hypertensive phenotypes are seen in studies on sheep exposed to dexamethasone during pregnancy (Dodic *et al.*, 1998; Gatford *et al.*, 2000). During the last trimester excess glucocorticoid exposure is sufficient to produce adult hypertension in rats (Levitt *et al.*, 1996) and also 'programmes' permanent

hyperglycemia and particularly, hyperinsulinemia in the adult offspring (Nyirenda *et al.*, 1998).

Studies suggest a molecular mechanism by which early life environmental factors may programme offspring physiology (Levine, 1957; Levine, 1962; Meaney *et al.*, 1988b; Meaney *et al.*, 1996b). Meaney *et al.* reported that 15 min of daily handling (NH) of rat pups during the first week or two of its life permanently increases GR density in the hippocampus and prefrontal cortex, but not in other brain regions (Meaney *et al.*, 1988a). This potentiates the HPA axis sensitivity to glucocorticoid negative feedback; keeping plasma glucocorticoid levels low throughout life, a state suitable for good adjustment to environmental stress (Meaney *et al.*, 1989; Meaney *et al.*, 1992). Similar effects were reported with neonatal glucocorticoid exposure as well (Catalani *et al.*, 1993).

The NH model appears to be of physiological relevance, since handling increases natural variation and maternal care-related behaviours and such naturally-occurring maternal behaviour also correlates similarly with hippocampal GR expression and the offspring HPA physiology (Liu *et al.*, 1997b). The long-term manifestations of some prenatal programming stimuli can be substantially modified by the immediate postnatal environment (Maccari *et al.*, 1995a), suggesting that distinct 'windows' occur. This shows that apparently similar early life events may produce different responses depending upon their degree, duration, developmental timing or sequence (Seckl and Meaney, 2004).

In other words in mothers who experience anxiety, the offspring may feel anxious or may be programmed to be anxious (Seckl, 2004). There are ample studies using rodent models of maternal stress which show substantial links between glucocorticoid exposure during gestation and unfavourable behavioural characteristics (Harris and Seckl, 2011). For example, Takahashi and colleagues compared prenatally stressed rats with non-stressed control rats and found that prenatally stressed rats exhibit impaired social behaviour and a reduced propensity to play (Takahashi *et al.*, 1992a). Depressive-like behaviours are also altered (Smith *et al.*, 2004; Cryan *et al.*, 2005; Cryan and Slattery, 2007).

In rodent models, prenatal stress increases immobility time in the Porsolt test and TST leads to anhedonia (Alonso *et al.*, 2000; Sobrian, 2000), effecting anxiety as well (Häuser *et al.*, 2009). It is reported that the offspring of prenatally stressed rats spend less time than controls in the open arms of an EPM (Vallée *et al.*, 1997; Estanislau and Morato, 2006; Murmu *et al.*, 2006) and rats from mothers that were prenatally stressed exhibit higher levels of anxiety than control rats in novel environments, such as the open field test (Takahashi *et al.*, 1992a; Ward *et al.*, 2000; Dickerson *et al.*, 2005).

A plausible alternative explanation for the increased 'hyperemotional' state of prenatally stressed rats is altered functioning of the amygdala (Johns *et al.*, 2005). The amygdala mediates fear, anxiety-related behaviour, learning and memory and thus, is instrumental for the expression of fear-conditioned learning (Davis, 1992). The amygdala contains MR, GR and CRF receptors and CRF producing cells (De Kloet *et al.*, 1998; De Kloet *et al.*, 2011).

Indeed, CRF may be the key neurotransmitter that mediates the effect of prenatal stress on anxiety. First, CRF levels are elevated in the central nucleus of the amygdala in prenatally stressed or glucocorticoid-treated rats (Cratty *et al.*, 1995; Welberg *et al.*, 2000; Welberg and Seckl, 2001). Second, CRF injections directly into the amygdala increase anxiety-related behaviour in rats (Dunn and Berridge, 1990).

Maternal stress during gestation can cause a range of long-term effects on the offspring (Weinstock, 2001). These include learning difficulties, anxiety, altered immune function, reduced attention, as well as changes to the cardiovascular responses to stress (Igosheva *et al.*, 2007) and hyperglycemia (Lesage *et al.*, 2004). Male and female offspring are different in these effects, it found a reduction in hippocampal plasticity in male, while in the female and this effect is protected from prenatal restraint stress (Darnaudéry and Maccari, 2008). Animal studies show that programming effects can last until the second generation at least. For example, Drake *et al.* (2005) found that when pregnant females were treated with dexamethasone, this reduced birth weight and caused glucose intolerance, which were transmitted to a second generation of offspring by both first generation female and male offspring (Drake *et al.*, 2005). Glover *et al.* (2010) suggested, the possibility that epigenetic changes can affect both oocyte and sperm is a controversial finding in biology (Glover *et al.*, 2010).

Other studies have shown how variation in the nature of maternal care can have long lasting effects on both function of the HPA axis and the behaviour of the offspring (Champagne *et al.*, 2004; Fish *et al.*, 2004; Cameron *et al.*, 2005;

Champagne and Meaney, 2006; Diorio and Meaney, 2007; Léonhardt *et al.*, 2007; Meaney *et al.*, 2007; Cameron *et al.*, 2008; Champagne *et al.*, 2008; Kappeler and Meaney, 2010)

1.8 MATERNAL BEHAVIOUR

1.8.1 DESCRIPTION OF RAT MATERNAL BEHAVIOUR

Numerous researches have comprehensively documented maternal behaviour with (the influence of oxytocin, prolactin, estradiol and progesterone priming her, the new dam is responsive to her pups from birth onwards (J.S. Rosenblatt, 1963). The litter sizes range from 8-16 pups and usually consist of an equal number of males and females.

During birth, the dam bites open the amniotic sac, eats the placentas and begins cleaning her pups as others emerge (Hudson *et al.*, 1999). Within 30 minutes after birth, the dam retrieves all of the pups and place them in the nest site, licks/grooms them and will adopt a nursing posture over them. Prior to weaning (which normally occurs between 21 and 31 day of age), all pups remain with the dam. As they mature, they start eating food crumbs from around the mother's mouth and fur and eventually, eat food at a distance from the nest and drink from a water bottle. Day by day, the pups spend less time in the nest with the dam and eventually the dam will move away from her offspring pups when they approach to suckle milk.

During the first 10 days postpartum, there are important maternal behaviours displayed intermittently by rat dams, including:

1.8.1.1 Retrieval

Pup retrieval occurs when the dams move her pups (by mouth) from one spot to another and inside the nest. This behaviour is observed during the early postnatal days, when pups get scattered around the nest, if the dam is suckling for instance and suddenly moves out of the nest a few pups attached to the teats get pulled from the nest and drop off some distance away. Normally dams retrieve their pups efficiently and quickly replace them in the nest; less maternal dams are slow to retrieve the pups. When the dam and pups are in the nest, she will frequently pick up/move pups and reposition them. This type of behaviour is called 'pick-ups' or 'mouthing' rather than true retrieval (Whishaw and Kolb, 2004).

1.8.1.2 Pup licking

An important source of stimulation for newborn pups is licking during the early postnatal period. A number of studies have confirmed the important effect of pup licking on the offspring's developing emotionality (Francis and Meaney, 1999), physiology (Kuhn and Schanberg, 1998) and cognition (Liu *et al.*, 2000b).

Pup body licking and pup anogenital licking are the two types of licking. Body licking is observed during nursing, before retrieval and between retrievals, whereas anogenital licking is observed while mothers nurse the pups (Whishaw and Kolb, 2004).

Moore (1984) reported that pups show reflexive extensions of limbs during anogenital stimulation. This plays an important role in sexual development especially in male pups, because that stimulation is important to ensure pup

defecation and urination. It also appears that the dams consume urine and faeces from their pups perhaps to maintain their own nutritional requirements during lactation (Whishaw and Kolb, 2004).

1.8.1.3 Nursing postures

Hovering and crouching are the two general types of nursing postures; Hovering is a posture when some or all of the pups are in the nest, the dam is positioned over them, but the dam is actually licking the pups, self grooming, moving the nest material or moving pups within the litter while hovering (Whishaw and Kolb, 2004). Meanwhile, crouching is considered to be a quiescent posture and usually occurs when the pups are sufficiently content. In this posture, the dam will stop other activities (e.g. moving the nest material, self grooming, or moving pups within the litter) and maintain herself in arched back position above her pups (Whishaw and Kolb, 2004). This posture is important helping to protect the pups from any environmental hazards, allowing them access to her teats for milk and regulating their temperature (Whishaw and Kolb, 2004).

1.8.1.4 Nest building

Nest building (in which the dam collects and gathers nesting materials to one site) is one of the maternal behaviours that is observed and recorded during a maternal behaviour test. Whishaw and Kolb, (2004) rated the nest building to five numbers; 1, no nest; 2, some material arranged in one location; 3, moderate, when low walls can be seen; 4, good nest, when walls are round and distinct; and 5, excellent nest, when walls of the nest are high and can take all the pups.

1.9 AIMS AND OBJECTIVES

1.9.1 BACKGROUND

Gestational Stress (GS) in rat dams causes behavioural effects (depressive-like behaviour, heightened anxiety-like behaviour and impaired cognitive function) and neuroendocrine changes such as impaired Hypothalamic-Pituitary-Adrenal (HPA) axis negative feedback in response to acute stress challenge, in the offspring at all postpartum ages tested to date). There are two possible mechanisms have been offered as explanations for how gestational stress induces these alterations in the offspring:

- 1- In *utero* exposure of the rat foetus to circulating maternal stress hormones that affect the phenotype of the offspring.
- 2- Reduced quality and quantity of maternal care shown by gestational stress dams that permanently alter the offspring. Maternal care occurs in nesting bouts during which the dams adopt nursing postures above (resembling a canopy) or beside (supine) their pups; lick and groom the ano-genital regions of the pups; and retrieve pups to the nest.

1.9.2 AIMS

1. We contend that disruptions of maternal behaviour caused by prenatal stress (PS) contribute to behavioural and physiological disturbances in the offspring. Furthermore, we speculate that impaired maternal behaviour in rats may model human postnatal depression and therefore, antidepressant agents should act to reinstate normal maternal behaviour in prenatally-stressed dams. Obviously, intrinsic changes in neurochemical and hormonal systems in both mother and

pup underlie PS effects; however, some of these disrupt mother-pup interactions and this contributes to the programming of offspring behaviour and physiology. Such interactions constitute a potentially valuable target for therapeutic intervention, but a number of issues need to be clarified and a detailed analysis of maternal behaviour following PS has to be made initially.

2. The change in maternal care following GS in the rat dam represents a form of postpartum depression, as it correlates with higher immobility scores in the Porsolt test. Administration of known antidepressant drugs to the dams should reduce these immobility times and simultaneously elicit increased maternal care.

1.9.3 OBJECTIVES

- 1- Is a period of stress during pregnancy sufficient to induce symptoms in the mother rat that model depressive states? We hypothesize that restraint stress; performed on a daily basis for the last half of the pregnancy should increase immobility (a measure of depressive-like behaviour) of the dams in a forced swim task.
- 2- If changes in rat maternal behaviour model human postnatal depression then antidepressant administration should act to normalize maternal behaviour in the rats. Using either a Serotonin Specific Reuptake Inhibitor (SSRI) such as sertraline, or a Tricyclic Antidepressant (TCA) such as imipramine, should reinstate normal maternal behaviour and act to offset the enduring effects of PS on the progeny. Antidepressants are efficacious in human depression and therefore they should ameliorate or diminish the impaired maternal behaviour of the rat.

3- To contribute further to the data suggesting that GS effect on maternal behaviour in the rat dam represents a novel and far more valid, model of human depression, than existing paradigms (such as olfactory bulbectomy). Moreover, to our knowledge, this is the first model that specially addresses post-partum depression, a condition that is poorly identified and one for which effective treatments have yet to be determined. In particular, if the model has predictive validity for antidepressant actions then this feature can be used to assess novel pharmacotherapies. Moreover, if the model shows predictive validity for antidepressant drugs, then it can replace current procedures such as the olfactory bulbectomy technique, which is invasive and has greater severity levels for the animals.

CHAPTER TWO: MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 ETHICAL APPROVAL

This project was carried out at the University of Bradford Biological Services Unit (BSU). All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986, under Home Office project licence No. 40/3415 and personal licence No. 40/10095 (Ali M. Alrumaih), following approval from the local ethics committee (see appendix). Housing, handling and testing of the animals were conducted according to Home Office regulations for investigations on live animals. These studies were funded by a Framework (FP7) European Union Grant (MOODINFLAME).

2.2 HOUSING AND HUSBANDRY

Lister hooded rats, male and female, were purchased from Harlan (UK) or Charles River (UK) for breeding. The change in supplier was due to an unexpected shortage of breeding stock from the primary source (Harlan, UK). Animals weighed between 120-260 grams upon arrival. The males and females were left to acclimatise in a designated holding room in the facility for one week, to overcome any transportation stress.

Male and Female rats were housed in groups of five (same sex) in standard, polycarbonate cages. The rats had free access to food (Harlan, UK), consisting of 18% protein in a compacted pellet and had ad libitum tap water. The bedding was Grade 6 woodchip, with additional sizzlenest nesting, plastic enrichment products and aspen chew blocks (Datesand LTD, Manchester). Housing conditions were strictly regulated; temperature was maintained at $22\pm 1^{\circ}\text{C}$, with

52±2 % humidity. The light/dark cycle was on from 7am until 7pm and off from 7pm. Cages were cleaned once a week by BSU animal technicians. However, when pups were born, cleaning of the cages was carried out by the experimenter, at appropriate times to reduce the impact of stress on the Dam and her litter.

2.3 MATING

Three female rats (12 weeks old, approximately 150g) were placed overnight with adult males (n=2) for the purpose of mating. The males and females remained together overnight and the females were checked the next morning for the presence of sperm (by smear test) or a vaginal plug.

2.4 SMEARING

To assess if mating had occurred each female rat underwent a vaginal smear test on the morning following being housed with a male(s).

The female was smeared using a small dropper with a rounded tip containing approximately 100 µl of warm water. The dropper tip was gently introduced into the opening of the vagina and the water was pumped into the vagina and immediately sucked back into the dropper by depressing one releasing a rubber bulb on the opposite end of the dropper. The sample was then aliquoted onto a glass slide. Using a microscope (Zeiss-Standard 20, Germany) at 400X magnification, slides were examined for the presence of sperm. If smears screened positive for sperm, females were re-housed in a new cage. Females giving a negative smear were returned to the mating cage.

Females with positive smears were designated as gestational day 0.

A few females had mucous plugs covering the vaginal opening indicating mating had occurred and were transferred to new cages without being smeared.

2.5 HOUSING OF PREGNANT RATS

Pregnant rats were group housed (n=5) into a fresh cage (60 × 80 × 20 cm³; Techniplast, UK) as detailed before.

The pregnant rats were left undisturbed until gestation day 10, at which time each animal was randomly assigned into either a control (non-stressed) or experimental (stressed) condition (Figure 2-1). Once they were assigned as control or stressed and had undergone the relevant procedure, animals were re-housed from the group cages into single cages, for the remainder of their gestational period. It is important to note that each pregnant Dam was assigned to a new cage, and identified only by a coded cage card such that all testing could be conducted in a 'blind' fashion. For each separate behavioural test another lab member would assign which animals were to be tested. The experimenter only had sight of the coded card and was unaware of which group, drug or day of gestation/postnatal time. All testing was done in a similar manner.

Note too that for the purposes of clarity in the remainder of this dissertation the word 'Dam' with a capital 'D' refer to the Dams in our studies. Use of 'dam' indicates animal mothers in other studies.

2.6 GESTATIONAL STRESS

2.6.1 PROCEDURE

For gestational days 10-20, pregnant rats were randomly assigned to be either a control or stressed animal (Figure 2-1). The females assigned as stressed were transferred from the maternity room to a procedure room and individually placed into clear perspex restrainer tubes (99mm x 320mm ,500gm model-AH002D, VetTech Solutions LTD), for 1h which usually occurred between (08:00 and 09:00 am). This chronic restraint stress technique was selected in view of its effectiveness in inducing overt neurobehavioural changes as reported in previous studies (Barlow *et al.*, 1978; Chapman and Stern, 1979; Ward and Weisz, 1984; Kinsley and Svare, 1986; Vallée *et al.*, 1997; Smith *et al.*, 2004; Darnaudéry and Maccari, 2008).

Each restrainer consists of a ventilated tube with one end sealed by an adjustable rubber blocker used to increase/decrease the horizontal length of the restraining area (useful for setting the restraint space according to the size of the rat) (Figures 2-2, 2-3). The animal was transferred into the restrainer through the open end of the tube, which was then shut with a guillotine-type door, restricting the rat in the confined space (Figures 2-2, 2-3). It should be noted that the animal was not immobilized by the restrainer, but confined to a tight space and visible at all times. The consensus opinion is that restraint increases the animals' degree of fear and sense of vulnerability, without causing physical discomfort or harm. The animal can turn around, groom and scratch, but generally remains motionless and appears lethargic. Urine drains out through the various ventilation openings that perforate the tube. Faeces tend to

collect within the tubes and the high degree of defecation is taken as a signal of the animal's heightened emotionality.

The females were placed into the restraint chamber during normal lighting and room conditions for 60 minutes (Figures 2-2, 2-3). During the restraint period, the rats were continually observed for any unusual behaviour, ensuring strict adherence for their welfare.

Once the procedure was completed, the Dams were returned to their single cages, restraining devices were washed and cleaned and the Dams were observed for 30 minutes for any subsequent abnormalities or behavioural disturbances.

The gestation period usually lasted for 21-22 days. Control, pregnant females were left undisturbed throughout their gestation periods (Figure 2-1), other than routine husbandry and weight monitoring.

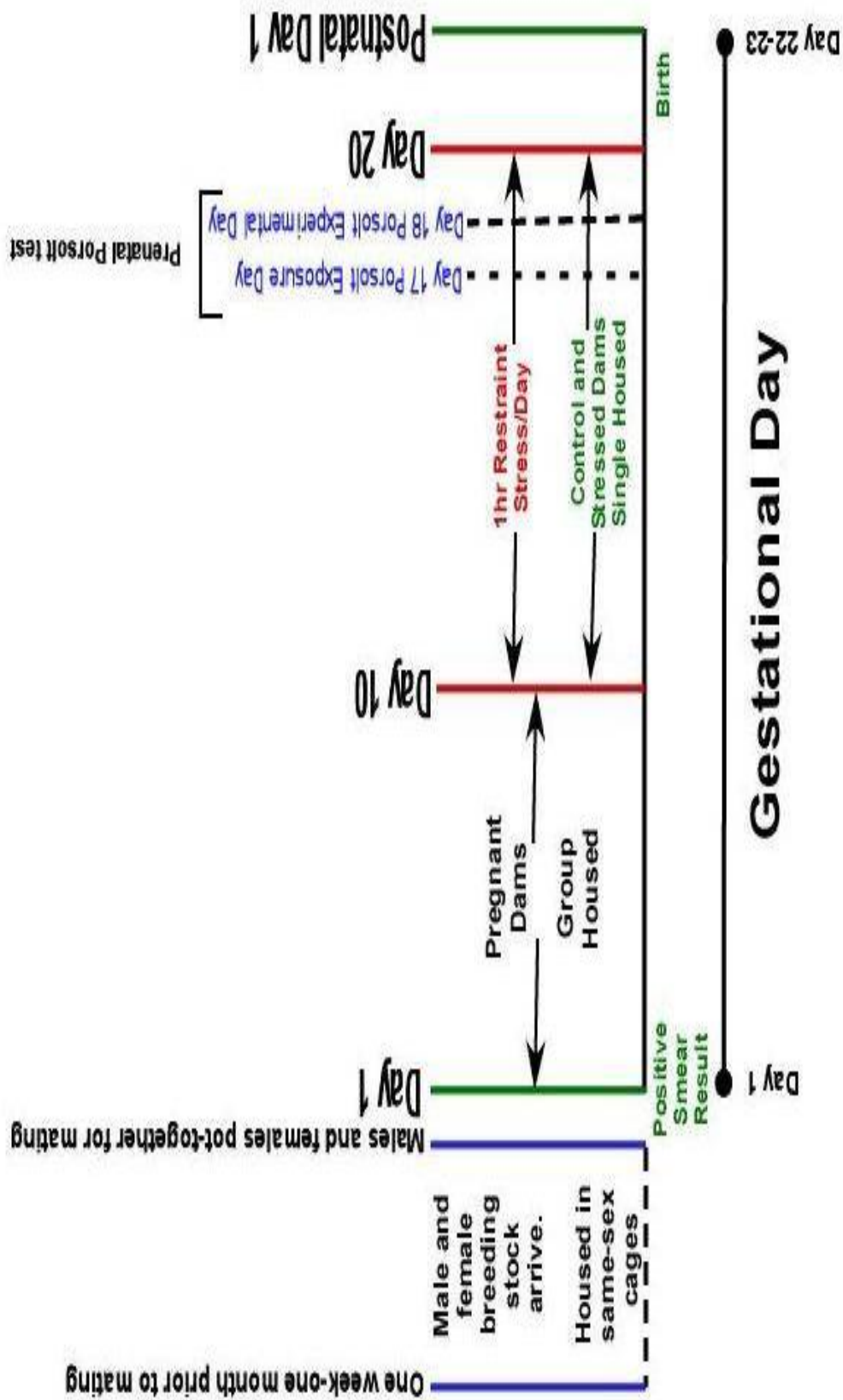


Figure 2-1: Diagram illustrating the sequences and procedures involved in the preparation of pregnant Dams, and the various manipulations which they were subjected to restrain stress.

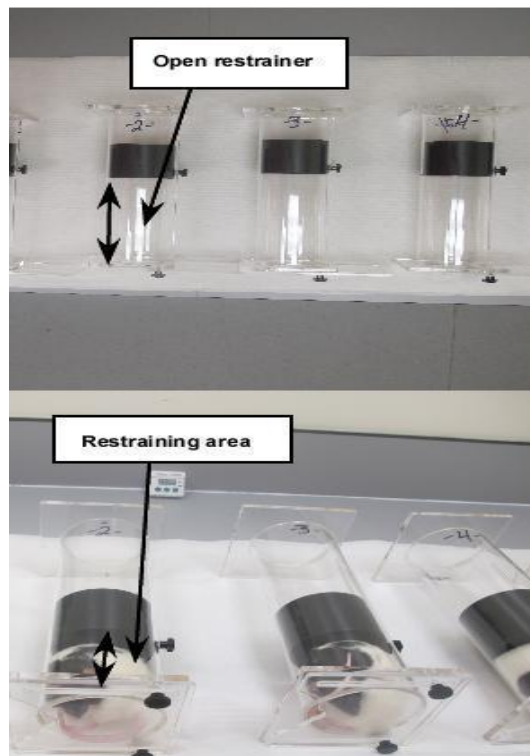


Figure 2-2: Pictures showing open restrainers and the restraining area after adjusting the rubber blocks.

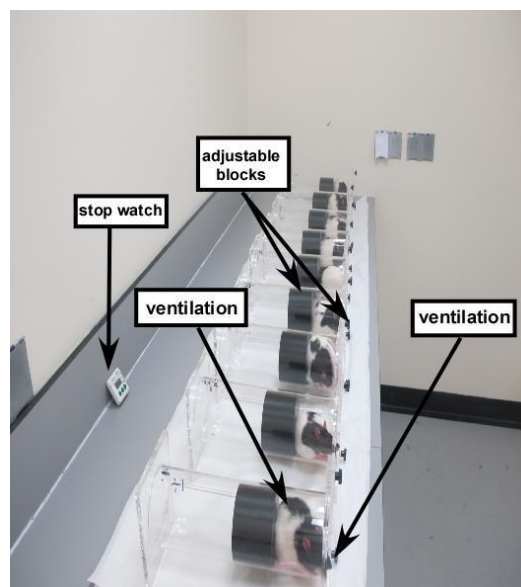


Figure 2-3: Picture showing pregnant rats undergoing restraint.

2.7 PRENATAL PORSOLT

2.7.1 PROCEDURE

Between days 17-20 of gestation, randomly selected control and stressed Dams underwent a Porsolt swim challenge (Figure 2-4) to assess immobility times. The test was conducted over two days; it consisted of an initial exposure day (day 17) for 15 minutes and, an experimental day for 5-10 minutes (day 18). On each occasion the water depth was adjusted for the size of the Dam such that the hind limbs could touch the bottom of the water chamber when fully extended and the animal could reach the water surface with its snout to breath. The overall chamber dimensions were 260X280X295mm (Height X Width X Diameter) (Figure 2-4). Pregnant rats were placed in the water chamber for the appropriate times and the total duration of immobility was recorded. The initial struggling activity that rats exhibit is eventually replaced by a periods of total immobility, which reflects learned helplessness'. The animals then cycle between active and passive states in an irregular fashion. The duration of immobility in stressed and control rats was recorded by the experimenter using a digital timer. The water temperature was maintained at 20-22⁰C these temperatures minimize immobility times and thus measures of immobility represent a more robust outcome (Smith *et al.*, 2004).

The procedure induces a fear-response, but this is minimised by employing a short duration exposure transient (Butler *et al.*, 1990; Vallée *et al.*, 1997; Korte, 2001; Korte *et al.*, 2007). All test sessions were recorded using Logitech® HD Webcam cameras C510 connected to computers and, immobility times also were recorded from the live images displayed on the computer monitors. This

technique reduces influences caused by the experimenters' presence (Figure 2-10). The camera was positioned 90cm above the chamber. After completion of the experiment, the animal was immediately placed into a bucket, under a heat lamp to aid drying and reduces hypothermia (Figure 2-5). The water in the Porsolt chamber was replaced after each test. Once the animals were completely warmed and dried, they were then transferred to their home cages and routinely monitored for residual signs of distress (e.g. lack of grooming, failure to feed, restlessness, timidity, or lethargy) for 60 minutes.

These animals were administered the peritoneal antidepressant or vehicle following birth, but neither they nor their progeny were used or tested in any further behavioural procedure. Tissues were harvested at weaning from the Dams; however, the pups were discarded. The exposure of the control Dams to the significant stress of the prenatal Porsolt test confounds any results, after birth because the control group is no-longer stress free and therefore results might be compromised.

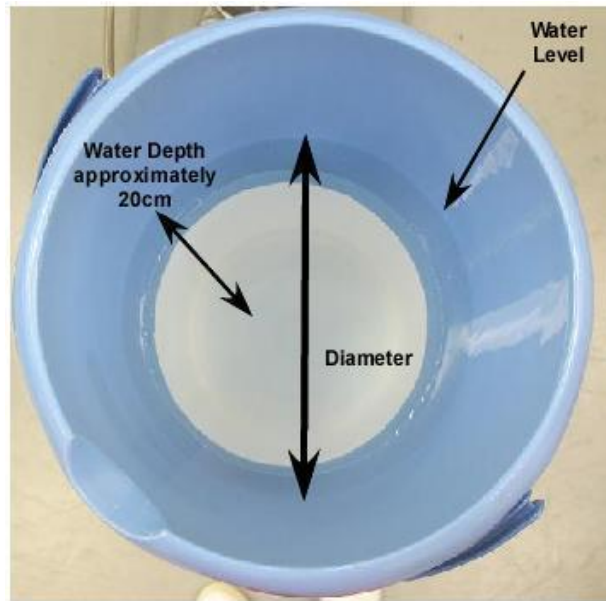


Figure 2-4: Picture showing water chamber used in Porsolt test.



Figure 2-5: Picture showing experimental animal under the heat lamp. Pregnant dam or offspring were kept for ten minutes to be dried completely.

2.8 EXPERIMENTAL GROUPS

2.8.1 MOTHERS

After the birth, the females were left for one day to acclimatise (day 0) and on day 1, litters were cross-fostered and culled to 5 males and 5 females. Different coloured bedding for stressed and control Dams assisted in coding the gestational treatment and with assessment of nest parameters (size, shape, quality and area of bedding) since the bright coloured material stands out from the typical brown materials. (1) a 20 grams brown colour, Grade 6 woodchip, with Sizzlenest nesting, plastic enrichment products and aspen chew blocks, all from Datesand LTD, Manchester and 5 grams of yellow 511 sizzle nest bedding from [SCA packaging stabox (The Netherlands)] for control (Figure 2-6) or (2) a 20 grams brown bedding and 5 grams of orange 048 sizzle nest bedding which [SCA packaging stabox (The Netherlands)] for stressed groups (Figure 2-7).

Control and stressed Dams were assigned to receive either vehicle (saline) or drug (sertraline /imipramine) on the day of birth.

This created 4 experimental conditions:

1. Control (C) + Vehicle (0.9% Saline) - This group (n=20) were left undisturbed during gestation and were given daily vehicle injections on postnatal days 1-10 (Figure 2-6).
2. Control (C) + Drug (imipramine hydrochloride-15mg/kg from Sigma Aldrich® or sertraline hydrochloride-10mg/kg from Tocris Bioscience) - These groups (n=13 imipramine) or (n=13 sertraline) (Figure 2-6), consisted of mothers that were left undisturbed during gestation and were given daily drug injections of either imipramine HCL or Sertraline HCL on each of postnatal days 1-10.

3. Prenatal Stress (PS) + Vehicle (0.9% Saline) - This group (n=20) consisted of prenatally stressed mothers that were given daily vehicle injections on postnatal days 1-10 (Figure 2-7).

4. Prenatal stress (PS) + Drug (imipramine hydrochloride-15mg/kg from Sigma Aldrich® or sertraline hydrochloride-10mg/kg from Tocris Bioscience - these groups (n=11 imipramine) or (n=11 sertraline) consisted of mothers that were stressed prenatally and administered imipramine HCL-15mg/kg or Sertraline HCL-10mg/kg on each of postnatal days 1-10 (Figure 2-7).

All injections were given as 1ml/kg intraperitoneally dissolve in 0.9% saline.

Drug doses were selected based on a wide survey of the literature, and represent mid-range concentrations. It is important in terms of animal usage and cost to test dose-response curves for this type of gestational work, and the current protocol methods existing research groups in the field.



Figure 2 6: Picture showing control mother with brown and yellow sizzle bedding.



Figure 2 7: Picture showing stressed mother with brown and orange sizzle bedding.

2.8.2 WEANING

At 21-22 days postnatally entire litters were re-housed into new cages for use in assessing prenatal stress and/or antidepressant effects on offspring behaviours. Offspring were tested in the elevated plus maze or the Porsolt swim test between 40-50 days of age, but each animal was tested only in one condition. Immediately following testing these offspring were killed and tissues were harvested.

Initially, the entire litters were transferred into the weaning cage, and the coded numbering system identifying gestation condition and postnatal drug treatment was copied onto the cage tags. As the offspring grew into juvenile and early adulthood (50-60 days of age), the male and female litters were devolved into 2 new cages. The same coding was maintained and the experimenter remained blinded to group identification. For a given Run, there would be similar, but not identical numbers of cages for offspring groups.

Any behavioural testing which entailed progeny of any or all 4 experimental groups was established as follows: an unblinded lab confederate identified the cage numbers that would be used to draw subjects from, ensuring that the appropriate experimental groups and sample sizes were evenly represented as much as possible. Furthermore, offspring were routinely selected from all cages such that individual litters, representing only a single Dam, were never over-represented in the behavioural tests.

2.8.3 OFFSPRING

The male and female offspring of stressed and control Dams formed 4 experimental conditions:

1. Control–Vehicle (CV) - This group consisted of the offspring (n= 79; Male = 39; Female = 40) born of mothers which were left undisturbed during gestation and had received daily injections of vehicle (0.9% Saline) on postnatal days 1-10 (Figure 2-6).
2. Control – Drug (CD) - This group consisted of the offspring (n=98; Male = 51; Female = 47) born of mothers which were left undisturbed during gestation and had received daily injections of imipramine hydrochloride-15mg/kg (Sigma Aldrich®) or sertraline hydrochloride-10mg/kg (Tocris Bioscience) on postnatal days 1-10 (Figure 2-6).
3. Prenatal stress – Vehicle (SV) - This group consisted of the offspring (n= 78; Male = 41; Female = 37) born of mothers which were stressed during gestation and had received daily injections of vehicle (0.9% Saline) on postnatal days 1-10 (Figure 2-7).
4. Prenatal stress – Drug (SD) - This group consisted of the offspring (n= 80; Male = 41; Female = 39) born of mothers that were stressed during gestation and had received daily injections of imipramine hydrochloride-15mg/kg (Sigma Aldrich®) or sertraline hydrochloride-10mg/kg (Tocris Bioscience) on postnatal days 1-10 (Figure 2-7).

2.9 POSTNATAL PORSOLT MOTHERS

2.9.1 PROCEDURE

Selected Dams were assessed using the Porsolt procedure at specific times following birth. The test was carried out over two days as before and consisted of the Dam being placed in the water chamber for 10 minutes as the exposure day and for 5 minutes on the experimental day 24hr later.

All parameters were observed as per the prenatal Porsolt test and immobility times were again recorded for each of the exposure and experimental days (Figure 2-8). Rats were only tested with the Porsolt procedure once, i.e. a pregnant Dam tested prenatally was not subjected to a postnatal Porsolt test. The Porsolt test was always administered to naive rats.

2.10 OFFSPRING PORSOLT

2.10.1 PROCEDURE

Selected offspring (males and females) were subjected to the Porsolt test at 40-50 days of age. The test was carried over 2 days, as before.

All parameters of the test were done as per the prenatal Porsolt test procedure.

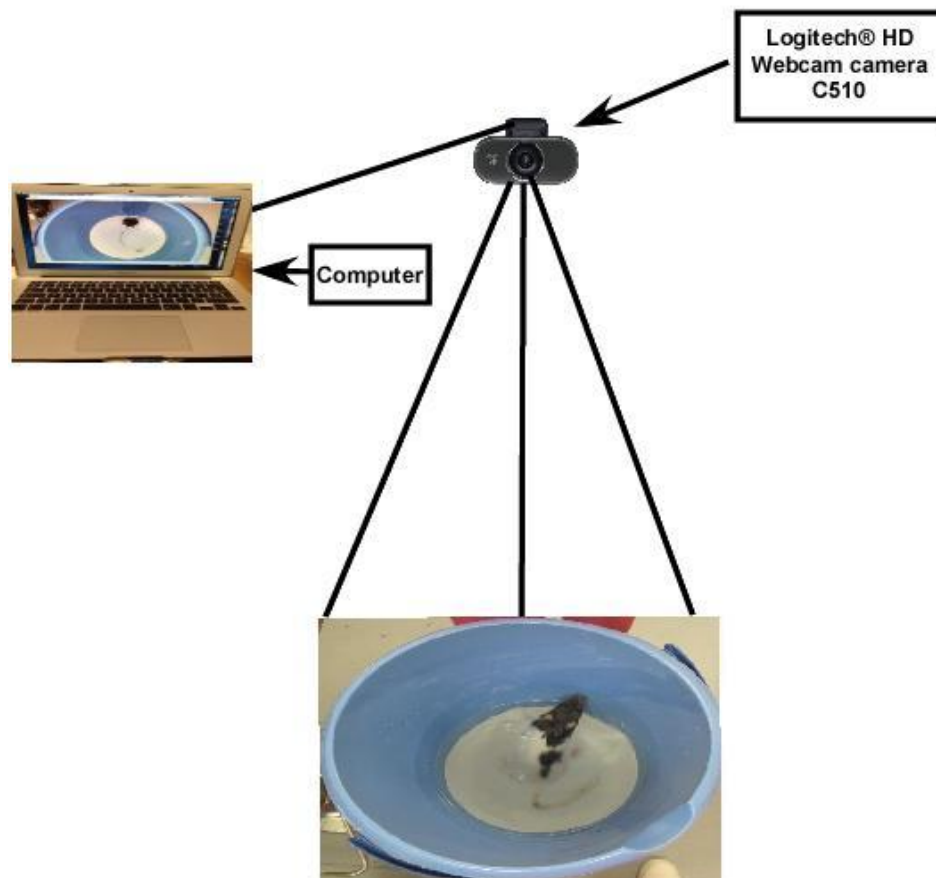


Figure 2-8: Schematic diagram of the Porsolt video recording system.

2.11 ELEVATED PLUS MAZE (EPM)

2.11.1 APPARATUS

The elevated plus-maze is comprised of two open arms without walls (10cm in width) and two closed arms (10cm in width) enclosed by walls (8.5cm) in height. These walls extended from a shared central point (10cm²) (Figure 2-9). Each arm is 44cm long. The apparatus is constructed from clear plexiglas and elevated to a height of 72cm above floor level. The floor of each arm is covered with a rubber mat to assist animals to walk without slipping. Rats are curious animals and will explore their environment (White, 1959; Martin and Bateson, 1993; Cavigelli, 2005); however, stressors often increase anxiety-like behaviour (Rodgers and Cole, 1993; Padovan and Guimarães, 2000; Adamec *et al.*, 2004). The amount to which the rat is willing to explore open arms is a measure of the rat's intrinsic anxiety (Pellow *et al.*, 1985; Pellow and File, 1986; Adamec *et al.*, 2004).

All test sessions were recorded using a Logitech® HD Webcam camera C510 connected to a computer, to minimise the influence caused by the presence of an experimenter and this was placed (90cm) above the apparatus.

2.11.2 PROCEDURE

On days 4 and 10 post-birth, selected Dams underwent anxiety assessment. The Dam was transported to the procedure room and placed on the centre point (facing an open arm) of the EPM. Rats from each condition were selected randomly for testing. In between each animal tested, the EPM was wiped cleaned with 70% ethanol (Sigma Aldrich®).

Entry into the open arms and closed arms was counted as defined by when the whole head and body of the rat were in that arm. However, if the rat fell from the platform, the trial time was stopped and resumed once the rat was replaced in the same position prior to the fall. The recordings were scored some later point by two individuals to ensure consistency. Times spent in the middle, central point, time spent in each arm and number of arm entries were recorded. The entire test trial lasted 5 minutes and each animal was exposed to the EPM only once.

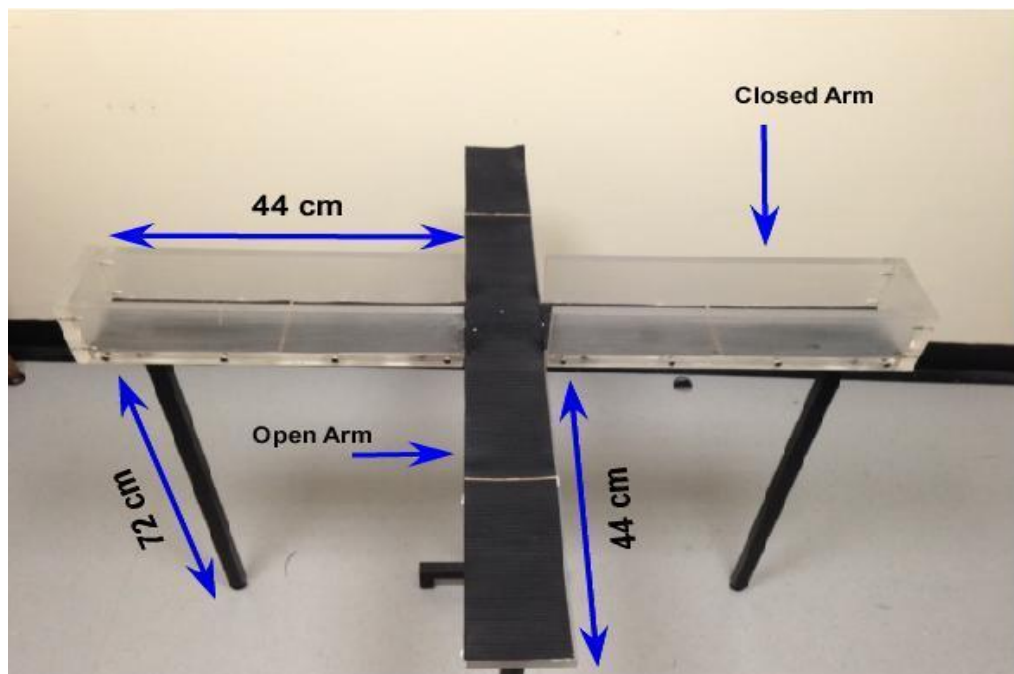


Figure 2-9: Picture showing the elevated plus maze with dimensions for open arms, closed arms and the height used for testing rat mothers and their offspring. Underneath the maze we positioned soft padding material to eliminate harm to any rat that fell off the maze.

2.12 MATERNAL BEHAVIOURS ASSESSMENT

On the second day after parturition, mothers were observed and recorded using a Logitech® HD Webcam camera C510 connected to a computer to preserve images of maternal behaviours of Dams that could be closely inspected at a later point. Electronic recordings also eliminated any interference or confounds caused by the presence of an experimenter, on any of the Dam's behaviours. The maternal behaviours were sampled for 30 min on any of postnatal days 1-10. The maternal cage was placed in a rack with the camera positioned above it.

The Dam was given 10 min to acclimatise to the new cage position. The recordings were carried out in the maternity holding room to minimize behavioural disturbances caused by cage transport, and external noises. To eliminate observational bias, the maternal behaviours from the videos were scored by two independent assessors blinded to the animal's treatment conditions. The following are descriptions of the various maternal behaviours examined;

Licking/Grooming Pups (LG): whether the mother was L/G the body or anogenital region of pups.

Arched Back Nursing (ABN); whether a Dam was nursing her pups with her back clearly arched and legs spread as in a canopy position or lying supine to suckle the pups (Figure 2-10, 2-11).

Behavioural measures were taken as the total time when the animal engaged in the particular behaviour during a 30 minute observation period. Scores from both observers were averaged to give an aggregate total time value.



Figure 2-10: Picture showing Arched back nursing (ABN).



Figure 2-11: Picture showing an example of supine nursing.

No contact; when the mother was absent from the nest (Figure 2-12).



Figure 2-12: Picture showing mother absent from her nest.

Retrieval of pups; where the mother picked/moved her pups by mouth from one spot to another in the cage (Figure 2-13).



Figure 2-13: Picture showing the mother retrieving a pup.

Nest building: whether a Dam was engaged building the nest or changing the architecture of the existing nest (Figure 2-14).

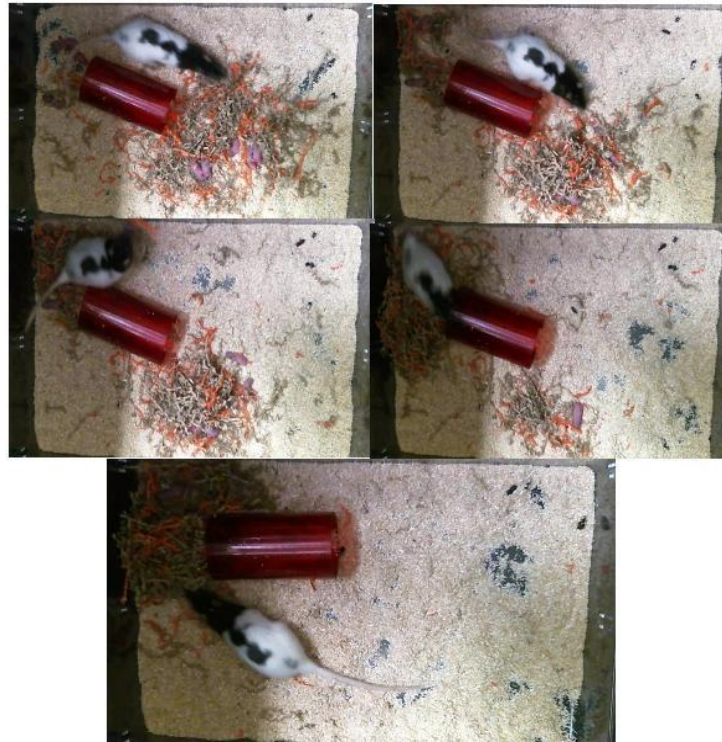


Figure 2-14: Picture showing mother were building her nest.

Time in the nest: total time that the mother spent inside the nest or in close contact with her pups.

2.13 ASSESSMENT OF MATERNAL NESTING

Every morning from PND2-10 cages were inspected for nest construction and pictures were taken.

In order to score nesting behaviour the following parameters were observed:

- i- Cages were arbitrarily divided into 5 quadrants to ascertain the position of the nest as: 1) Top Left; 2) Top Right; 3) Bottom Right; 4) Bottom Left; 5) ; and Centrally Located nest (Figures 2-15 and 2-16).
- ii- Amount of nesting materials used over a 10 day period.
- iii-Frequency of nest moves.



Figure 2-15: Picture showing the quadrant divisions.
1 as top left, 2 as top right, 3 as bottom right, 4 as bottom left, and 5 as central of the cage

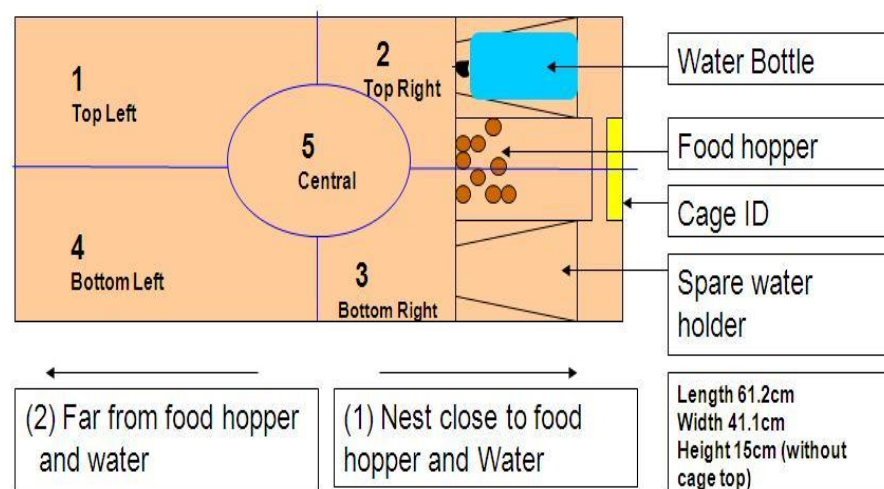


Figure 2-16: Diagram illustrating the Cage specifications. it shows 1 as top left, 2 as top right, 3 as bottom right, 4 as bottom left, and 5 as central of the cage ,numbers 1 and 2 assigned as the distance from water and food hopper wither closed or far.

2.14 STATISTICAL ANALYSIS

All statistical analyses were carried out using the statistical package StatView® (SAS Institute.) and Excel® program to produce graphs.

We have the prenatal condition as one variable (Control or Stressed) and another variable for treatment (Vehicle or Drug), where appropriate Day of testing appears as a repeated-measures variable, for instance maternal behaviours was examined on select postnatal days to determine if either prenatal condition or postnatal treatment effects emerged or diminished as the pups grew.

Significant main and/or interaction effects were examined using a *posteriori* tests using Bonferroni-corrected f-tests maintaining the experiment-wise alpha level equal to $p < 0.05$.

Data for Dams and offspring were managed the same, save to note that for the offspring prenatal condition and postnatal drug treatment refers to the mothers of the offspring.

CHAPTER THREE: RESULTS

3. RESULTS

3.1 EFFECTS ON WEIGHTS

3.1.1 EFFECT OF PRENATAL STRESS ON RAT GESTATIONAL WEIGHTS

Figure 3-1 shows the effects of prenatal stress on Dam weights for gestation days 11-19. A repeated measures ANOVA showed that non-stressed Dams gained significantly more weight over the 8 days in comparison to the stressed Dams ($F(7,364)=2.82, p<0.045$).

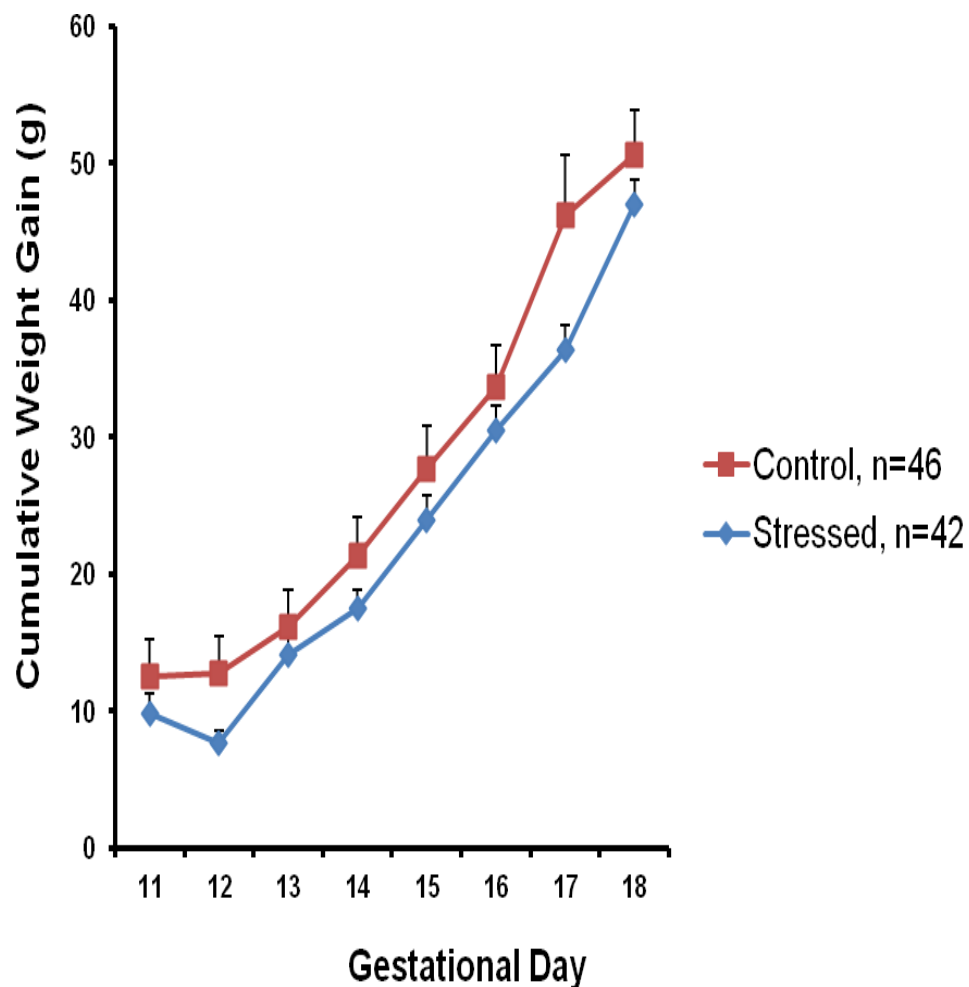


Figure 3-1: Mean Cumulative weight gain (g) during gestational days 11-19, for control and gestationally-stressed Dams.
Data shown are Means \pm SEM.

3.1.2 POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF SERTRALINE

Figure 3-2 depicts postnatal Dams' weight measured over postnatal days 1-10. An ANOVA on the postnatal Dams' weights revealed no effect of prenatal condition with ($F(1,200)=0.01$, $p<ns$). As can be seen in Figure 3-2 below, the weight over days 1-10, in aggregation shows that stressed Dams have a slower weight gain than that of the control Dams. A similar ANOVA examining the effects of drug treatment (sertraline, 10mg/kg) on postnatal weight gain of Dams, revealed a significant effect of sertraline with ($F(1,200)=7.62$, $p<0.01$). An ANOVA examining the interaction between drug treatment (sertraline, 10mg/kg) and prenatal condition revealed a significant interaction with ($F(8,200)=3.20$, $p<0.002$).

As can be seen in Figure 3-2, all Dams showed modest weight gains over postnatal days 1-10, but the magnitude of this effect was reduced in all animals administered sertraline.

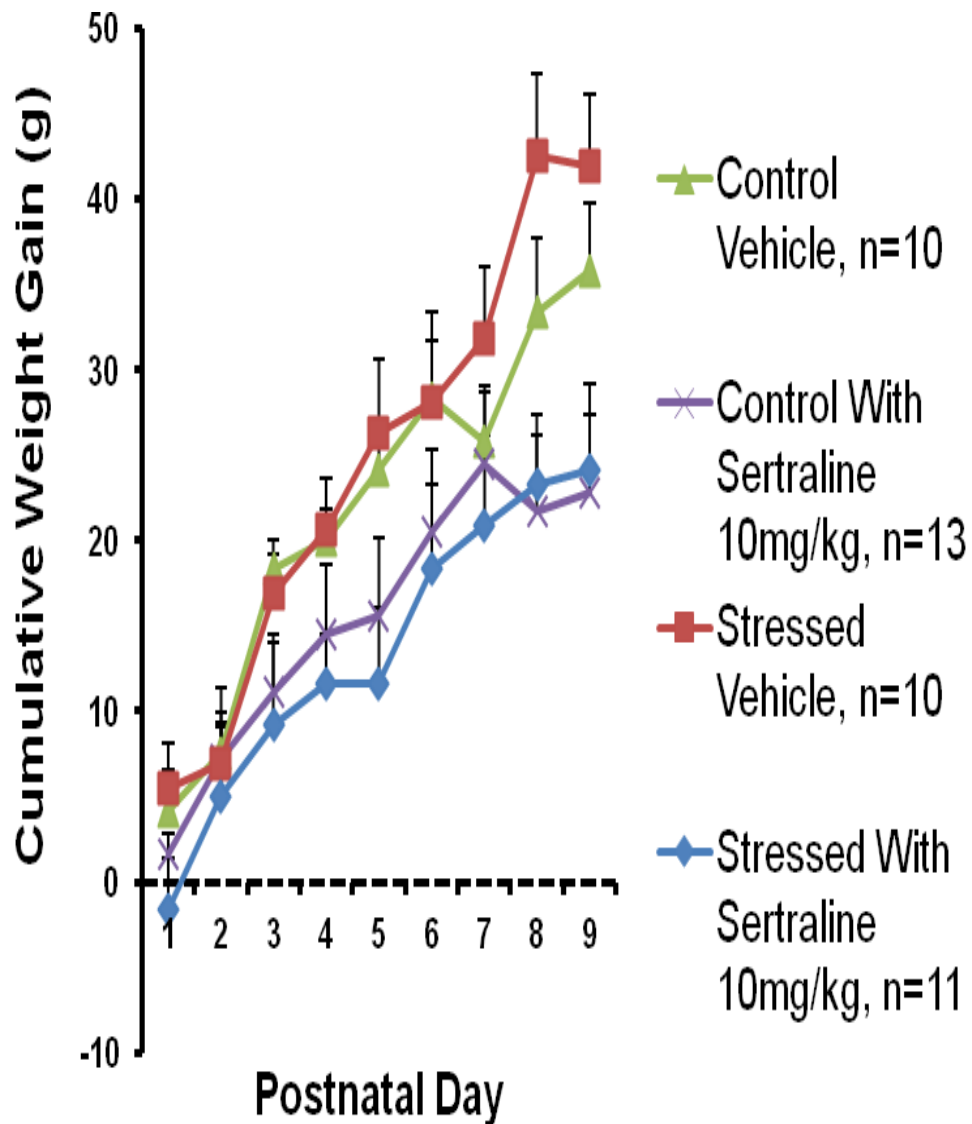


Figure 3-2: Mean postnatal cumulative weight gain (g) of Dam's days, 1-10 after giving birth. Data represent the mean weight gain (cumulative) for Dams who had undergone gestational restraint stress, and non-stressed controls. Sertraline (10mg/kg) or vehicle (saline). Data shown are means \pm SEM for each group of Dams.

3.1.3 POSTNATAL DAM WEIGHTS AND THE EFFECTS OF IMIPRAMINE

Figure 3-3 depicts postnatal Dams' weight measured on postnatal days 1-10. An ANOVA on the postnatal Dams weights showed no effect of prenatal condition with ($F(1,43)=0.49$, $p<ns$). As can be seen in Figure 3-3 below, the weights over days 1-10, in aggregation show that stressed Dams have a slower weight gain than that of the control Dams. A similar ANOVA examining the effects of drug treatment (imipramine, 15mg/kg) on postnatal weight gain of Dams, revealed a significant effect of imipramine with ($F(1,43)=20.12$, $p<0.0001$). An ANOVA examining the interaction between drug treatment (imipramine 15mg/kg) and prenatal condition revealed a significant effect on Dam weights with ($F(8,344)=5.67$, $p<0.0001$).

In contrast to the sertraline treatment, those Dams receiving imipramine showed negative weight gain over postnatal days 1-10 and only achieved basal weight levels at 10 days. This effect was slightly more pronounced in the stressed Dams.

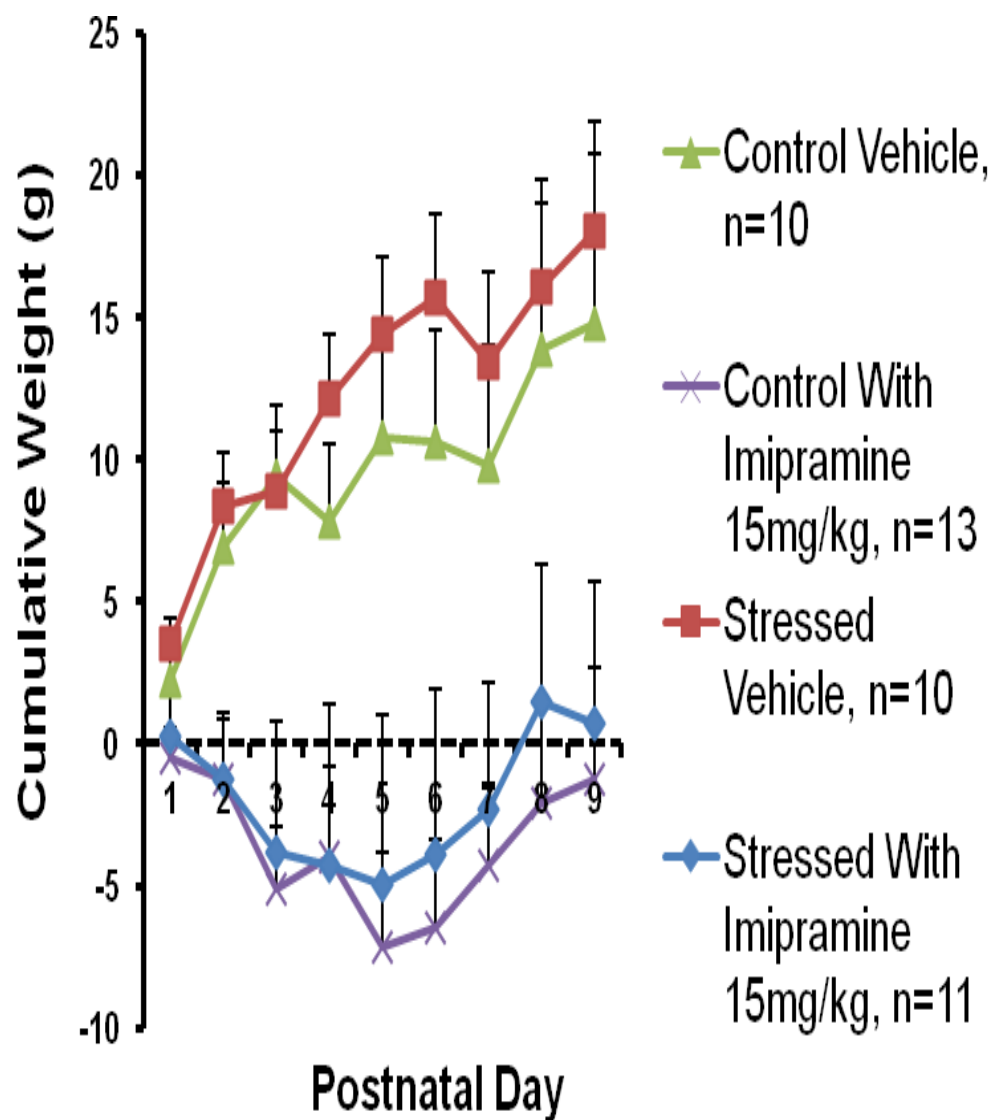


Figure 3-3: Mean postnatal cumulative weight gain (g) of Dams from days 1-10. For those which had undergone gestational restraint stress and non-stressed controls on imipramine (15mg/kg) or vehicle (saline).

3.1.4 OFFSPRING WEIGHT AND THE EFFECTS OF SERTRALINE

Figure 3-4 shows litter weights measured from postnatal day 1-10. An ANOVA on the postnatal litter weights revealed a main effect of prenatal condition with ($F(1,40)=3.56$, $p<0.05$). As can be seen in Figure 3-4 below, the aggregated weight over days 1-10, shows that litters from stressed Dams have a slower weight gain than litters from the control Dams. A similar ANOVA examining the effects of sertraline (10mg/kg) postnatally on Dams' litter weights, revealed no significant effect of sertraline on litter weight gains with ($F(1,40)=1.31$, $p<ns$). An ANOVA examining the interaction between drug treatment (sertraline, 10mg/kg) for Dams postnatally and their prenatal condition revealed no significant interaction with ($F(8,320)=2.51$, $p<ns$).

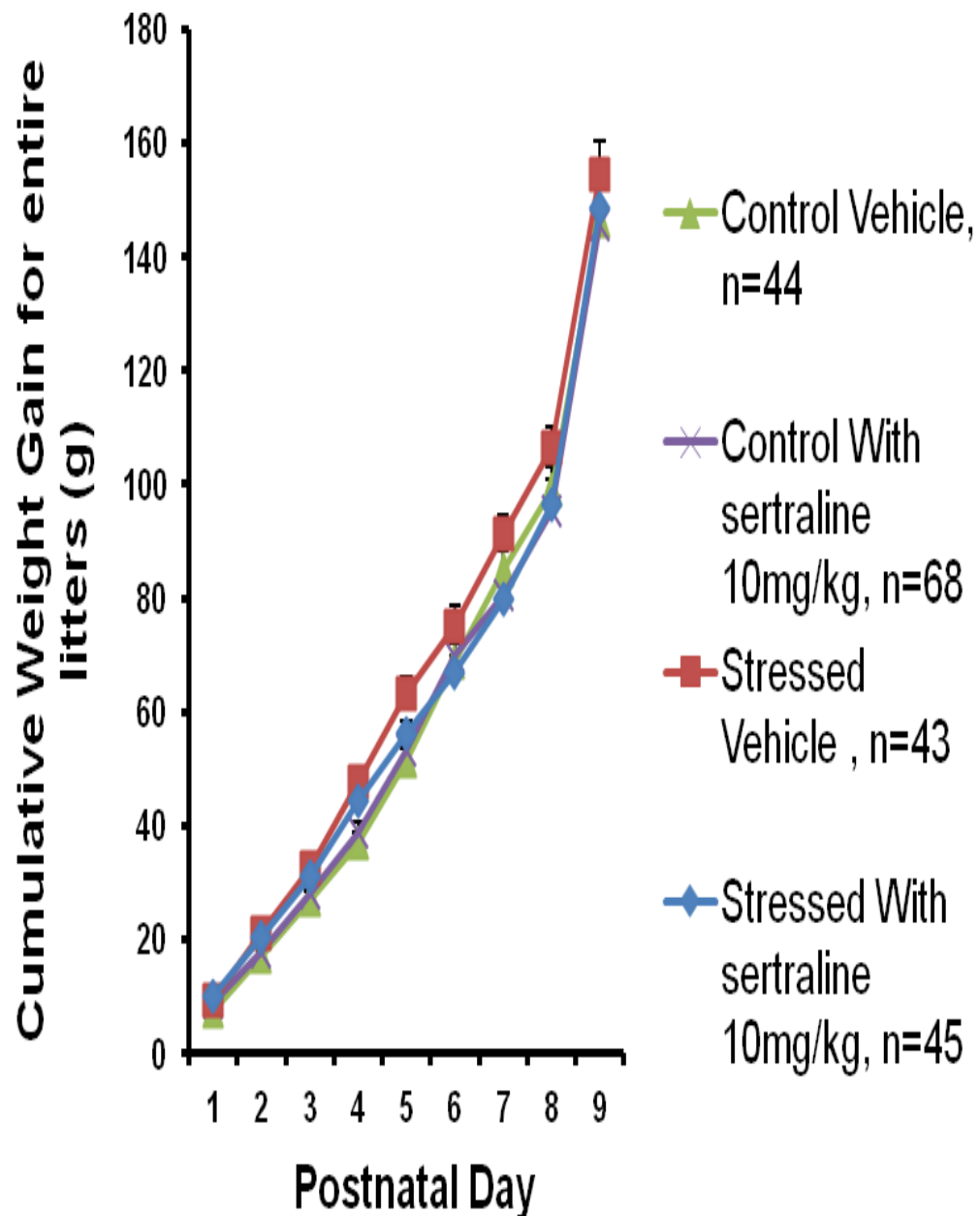


Figure 3-4: Mean pups cumulative weight gain (g) from days 1-10. From stressed and non-stressed Dams on either drug sertraline (10mg/kg) or vehicle (saline). Data shown are Means \pm SEM.

3.1.5 OFFSPRING WEIGHTS AND THE EFFECTS OF IMIPRAMINE

Figure 3-5 shows litter weights measured from postnatal day 1-10. An ANOVA on the postnatal litter weights revealed no effect of prenatal condition with ($F(1,44)=2.649$, $p<ns$). As can be seen in Figure 3-5 below, litter weight over days 1-10 show that stressed Dams' litters have a slower weight gain than those litters from control Dams. A similar ANOVA examining the effects of imipramine (15mg/kg) administered postnatally to the Dams revealed a significant effect of imipramine on litter weights with ($F(1,44)=9.37$, $p<0.003$). An ANOVA examining the interaction between drug treatment (imipramine, 15mg/kg) for Dams postnatally and prenatal condition revealed a significant effect interaction with ($F(8,352)=14.46$, $p<0.0001$).

Again, it is obvious that litters from Dams administered imipramine showed diminished weight gains for most of postnatal days 1-10, although by postnatal day 10, there was a tendency for all litters to be similar in weight.

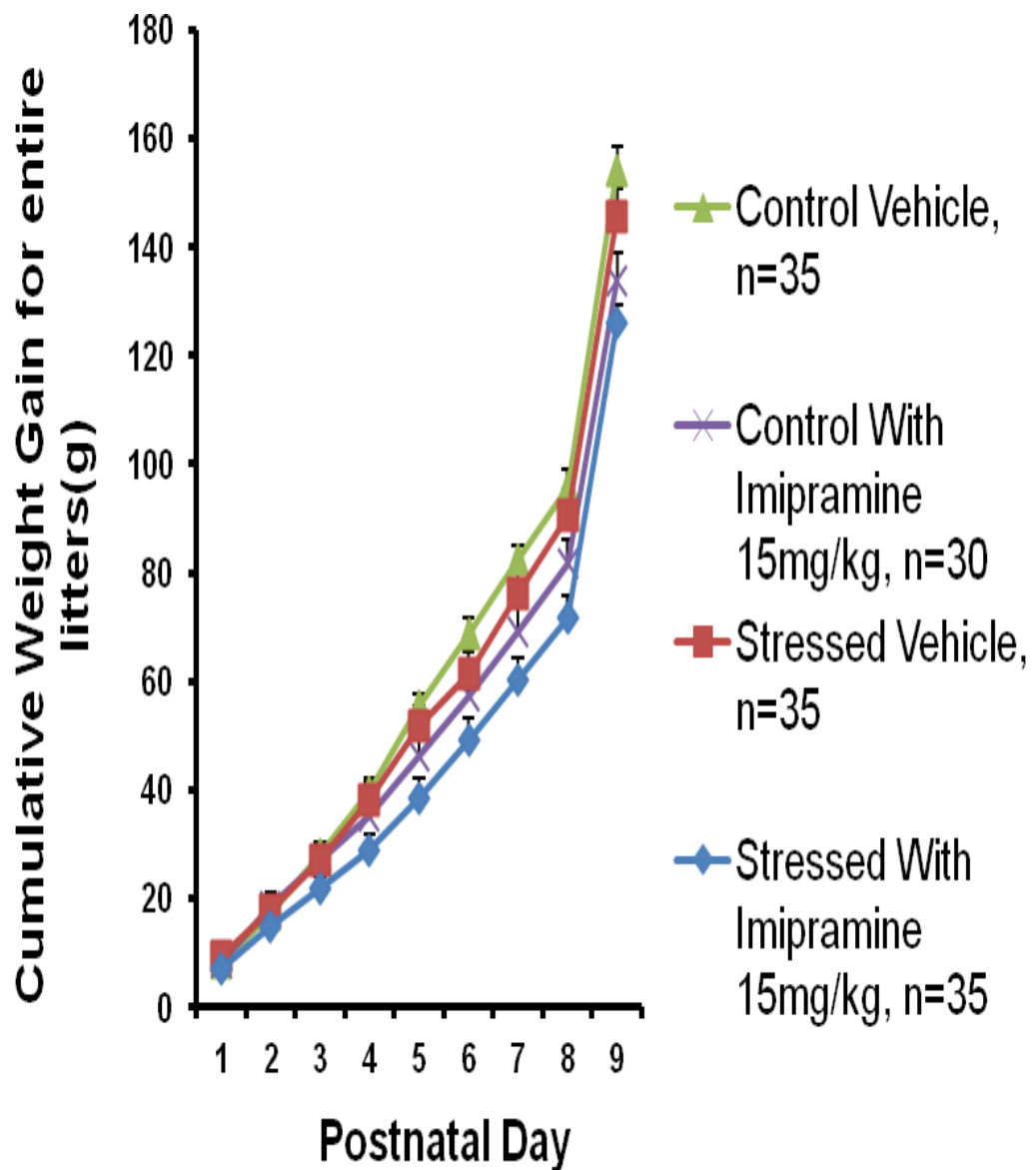


Figure 3-5: Mean pups cumulative weight gain (g) from days 1-10. From stressed and non-stressed Dams on either imipramine (15mg/kg) or vehicle (saline). Data shown are Means \pm SEM.

3.2 PRENATAL PORSOLT TEST IN PREGNANT DAMS

Figure 3-6 shows the immobility times recorded from pregnant Dams on gestation Day 17 (Exposure day-15 minutes) and Day 18 (Experimental day 5-10 minutes). An ANOVA comparing Control and Stressed Dams on the Day 17 immobility data revealed a significant effect with ($F(1,39)=4.02, p<0.05$).

A similar ANOVA for the Day 18 immobility data showed no significant effect with ($F(1,39)=2.09, p<ns$). The data are illustrated in Figure 3-6 below.

Overall, there was a tendency for stressed Dams to show greater immobility in the Porsolt test, an effect that achieved statistical significance for gestational day 17.

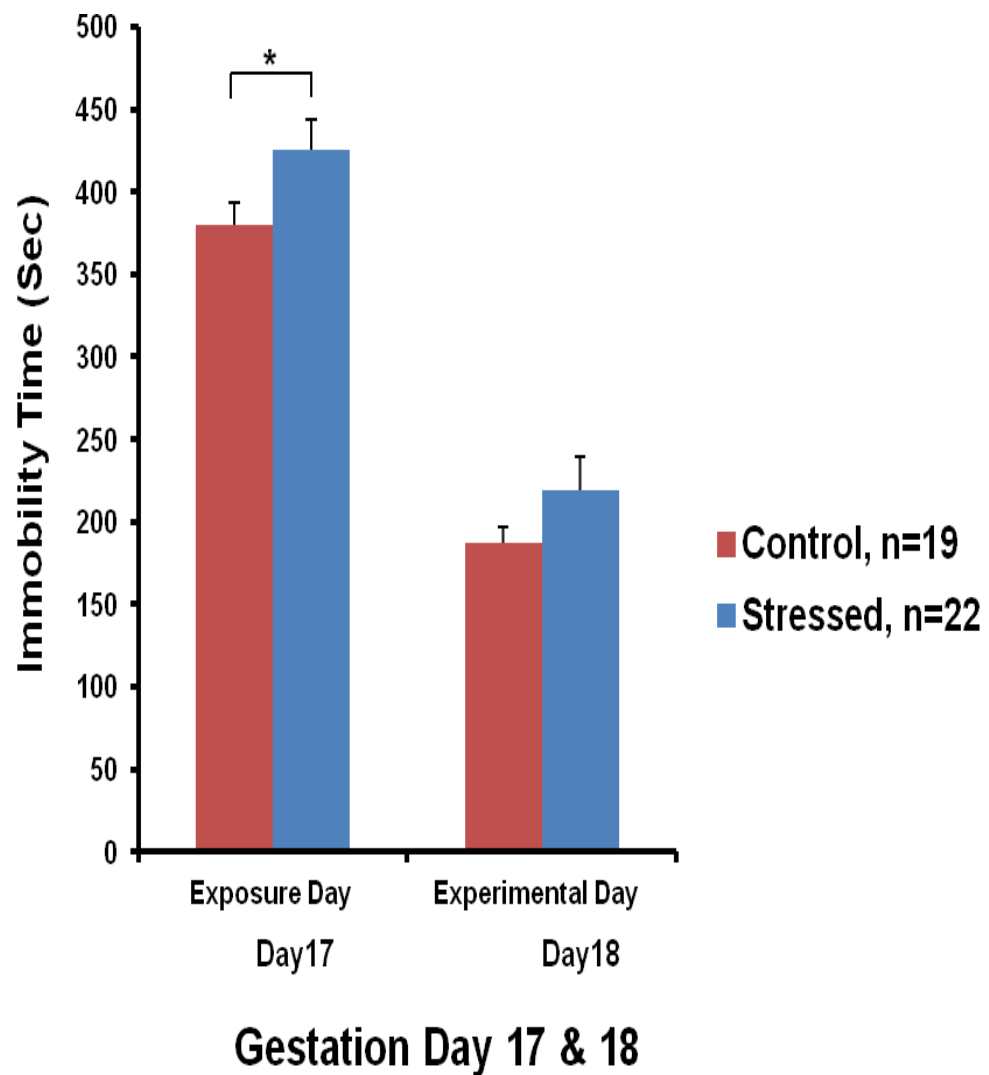


Figure 3-6: Shows the Prenatal Porsolt data from control and stressed Dams at Days 17 and 18 of gestation (Exposure Day and Experimental Day). Data shown are Means \pm SEM.

*Significantly different, $P < 0.05$, Bonferroni t-test.

3.3 POSTNATAL PORSOLT DATA FOR DAMS AND THE EFFECTS OF IMIPRAMINE

Postnatal Porsolt testing was done on Dams at postnatal day 9 (Exposure day - 10 min) and day 10 (Experimental day-5 min). An ANOVA on the data for the Exposure Day revealed no significant effect of prenatal condition with ($F(1,33)=0.006$, $p<ns$). An ANOVA on these data revealed no significant effect of postnatal drug treatment (imipramine 15mg/kg) on immobility times with ($F(1,33)=1.1$, $p<ns$). An ANOVA examining the prenatal condition by postnatal imipramine (15mg/kg) interaction revealed no significant effect on immobility times with ($F(1,33)=1.13$, $p<ns$).

Figure 3-7 shows that stressed Dams given imipramine (15mg/kg) exhibited higher immobility times, but this was not significant in comparison with the other groups.

An ANOVA on the data for the Experimental Day revealed no significant effect of prenatal condition on immobility times with ($F(1,33)=1.05$, $p<ns$). An ANOVA on these data revealed a significant effect of postnatal imipramine (15mg/kg) treatment on immobility times with ($F(1,33)=4.28$, $p<0.05$). An ANOVA examining the prenatal condition X postnatal imipramine (15mg/kg) interaction revealed no significant effect on immobility times with ($F(1,33)=0.26$, $p<ns$).

Figure 3-7 shows that stressed Dams who had been given imipramine and exhibited reduced immobility times in comparison with non-drug treated groups.

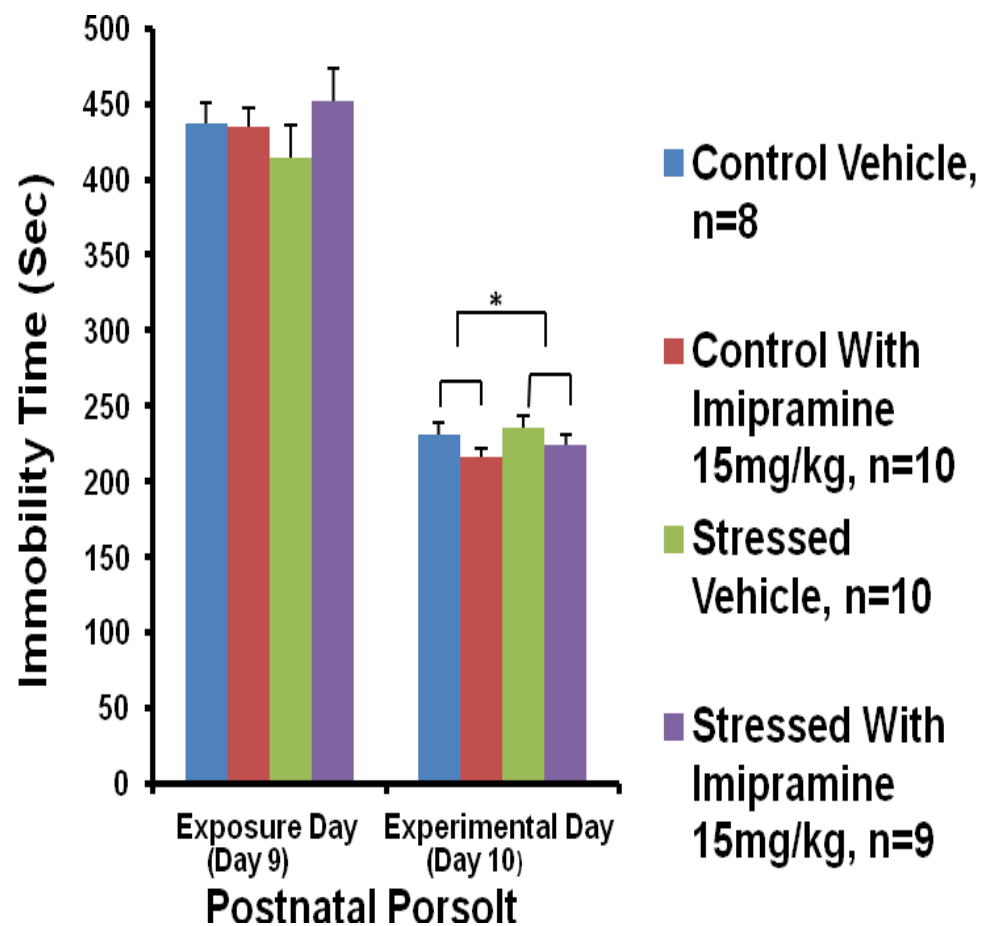


Figure 3-7: Shows data for the control and stressed Dams following birth. Dams were treated with imipramine (15mg/kg) administered daily following birth. Data shown are Means \pm SEM.
 *significantly, different from same group animals were given saline, $p < 0.05$, Bonferroni t-test.

3.4 OFFSPRING PORSOLT AND THE EFFECTS OF IMIPRAMINE

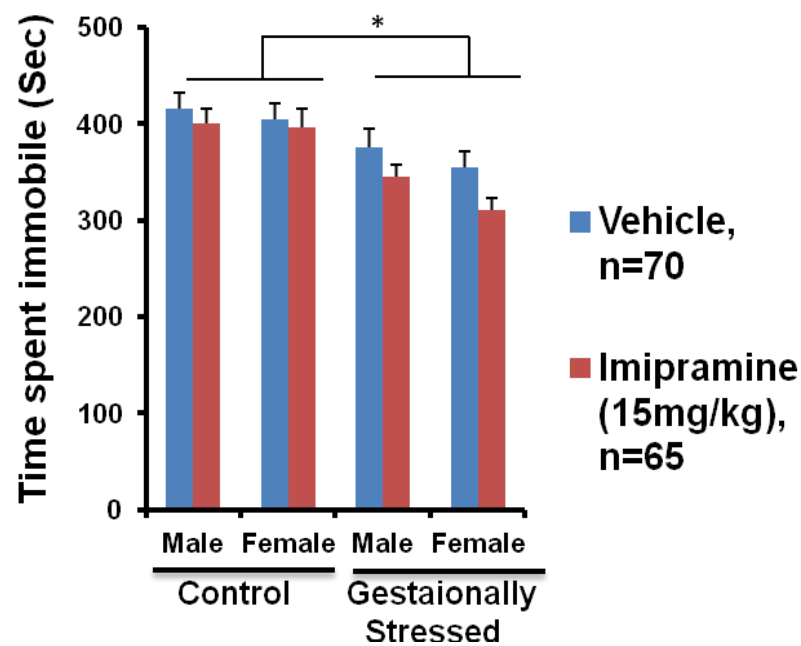
Figure 3-8 illustrated the data obtained for the Porsolt test in offspring of gestationally-stressed and control rat Dams. After giving birth, Dams were treated with saline (vehicle) or imipramine (15mg/kg) daily for 10 days. Offspring were weaned on postnatal days 20-21 and tested at 40-50 days of age. Panel A shows immobility times for male and female offspring recorded at the initial test (the Exposure Day), while Panel B reveals the immobility times recorded for the second day of testing (the Experimental Day). Note that the Exposure Day trial is 10min in duration, while the Experimental Day trial test is only 5min.

An ANOVA on the data for the Exposure Day revealed a significant effect of prenatal condition on immobility times with ($F(1,127)=25$, $p<0.0001$), which shows that offspring of stressed Dams exhibited lower immobility times in comparison to those offspring of control Dams. There was also a significant effect of postnatal drug treatment on immobility times with ($F(1,127)=4.5$, $p<0.04$), this significant effect was not altered by postnatal imipramine treatment, although imipramine itself decreased immobility times in both control and stressed offspring. Moreover, there was no significant effect of gender (male or female) with ($F(1,127)=2.45$, $p<ns$). There was no significant effect of prenatal condition X postnatal drug treatment X gender immobility times interaction for these data ($F(1,127)=0.23$, $p<ns$).

An ANOVA on the data for the Experimental Day as shown in Panel B, revealed a significant effect of prenatal condition on immobility times with ($F(1,127)=14.4$, $p<0.0002$), which showed that offspring of stressed Dams exhibited lower

immobility times in comparison to those of offspring from control Dams. However, there was no significant effect of postnatal drug treatment on immobility times with ($F(1,127)=1.65$, $p<ns$). Moreover, there was a significant effect of gender (male or female) with ($F(1,127)=3.5$, $p<0.05$), which showed that males and females of prenatally-stressed Dams exhibit lower immobility times in comparison to those of offspring from control Dams. There was a significant effect of prenatal condition X postnatal drug treatment interaction for these data with ($F(1,127)=6.4$, $p<0.01$). There was no significant effect of prenatal condition X postnatal drug treatment X gender immobility times interaction for these data with ($F(1,127)=0.46$, $p<ns$), this showed that imipramine treatment decreased immobility times in gestationally stressed offspring.

A. Exposure Day (10min)



B. Experimental Day (5min)

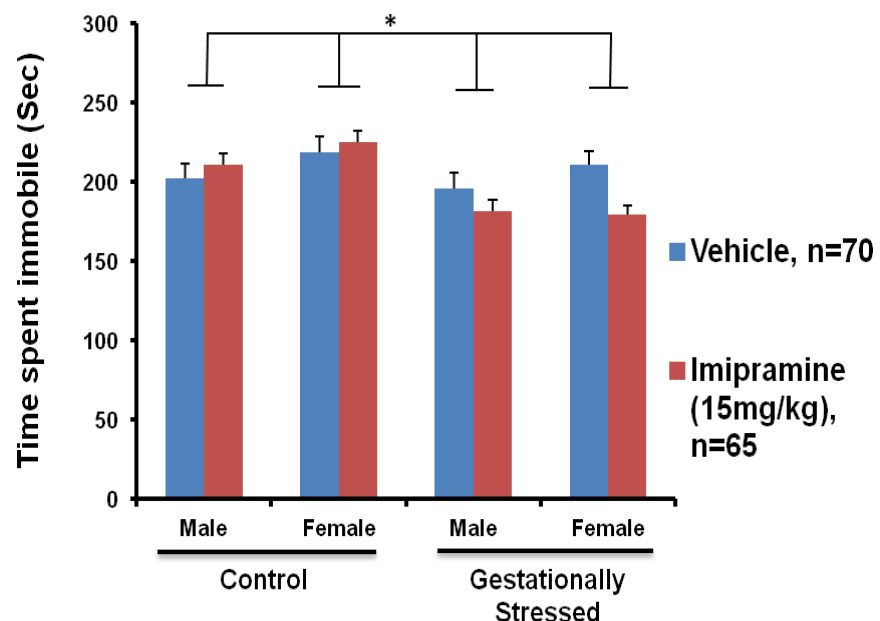


Figure 3-8: Immobility times for the Porsolt task recorded from the offspring (male and female) of gestationally stressed or control rat Dams following postnatal drug or vehicle treatment of the Dams immediately after birth.

Offspring were tested on 2 consecutive days (Exposure and Experimental Days) after they had reached adolescence (40-50 day old).

Data shown are Means \pm SEM.

* Significantly differences between offspring of control and gestationally stressed Dams and significant differences between vehicle and imipramine-treated animals, $p < 0.05$, Bonferroni t-test.

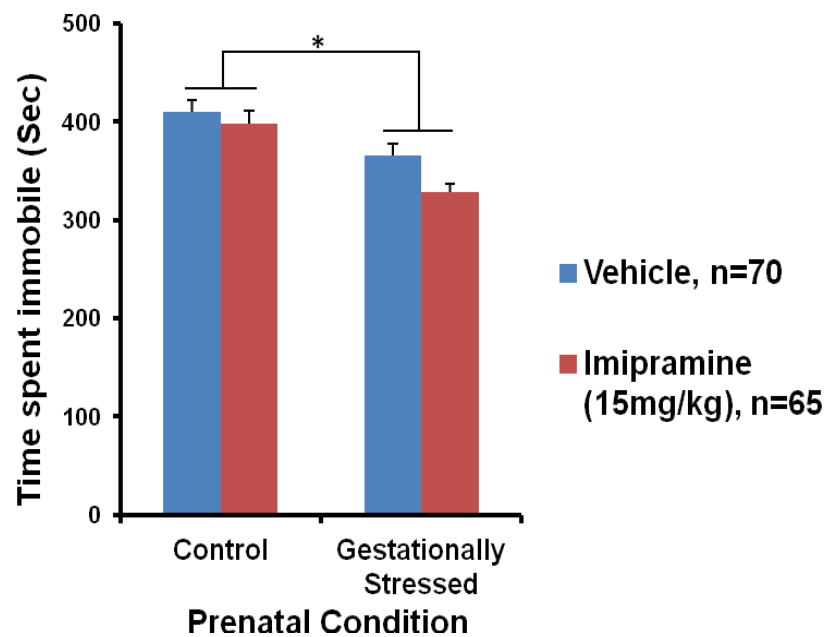
Figure 3-9 shows the immobility times for offspring of control and prenatally-stressed Dams subjected to the Porsolt test at 40-50 days of age, but removing the variable of offspring gender. An ANOVA on the data for the Exposure Day revealed a significant effect of prenatal condition on immobility times with ($F(1,131)=24.82$, $p<0.0001$), which showed offspring's of prenatally-stressed Dams exhibit lower immobility times in comparison to those offspring from control Dams. Moreover, there was a significant effect of postnatal drug treatment on immobility times with ($F(1,131)=4.5$, $p<0.004$), which shows that postnatal imipramine treatment decreased the immobility time in offspring from prenatally-stressed and control Dams. There was no significant effect of prenatal condition X postnatal drug interaction for these data with ($F(1,131)=1.14$, $p<ns$).

Figure 3-9 shows that offspring of stressed Dams exhibited lower immobility times in comparison with those of offspring of control Dams. This difference was not altered by postnatal imipramine treatment, although imipramine itself decreased immobility time in both control and stressed offspring.

An ANOVA on the data for the Experimental Day Porsolt procedure (Panel B) revealed a significant effect of prenatal condition with ($F(1,131)=14.22$, $p<0.0002$), which showed that offspring of prenatally-stressed Dams exhibited lower immobility times compared to control Dams. An ANOVA on these data showed no significant effect of postnatal drug treatment with ($F(1,131)=1.54$, $p<ns$). An ANOVA examining the interaction between prenatal condition and postnatal drug treatment showed a significant effect with ($F(1,131)=6.33$,

$p < 0.01$), showing imipramine reduced immobility times and effect that was evident only for the offspring of stressed Dams.

A. Exposure Day (10min)



B. Experimental Day (5min)

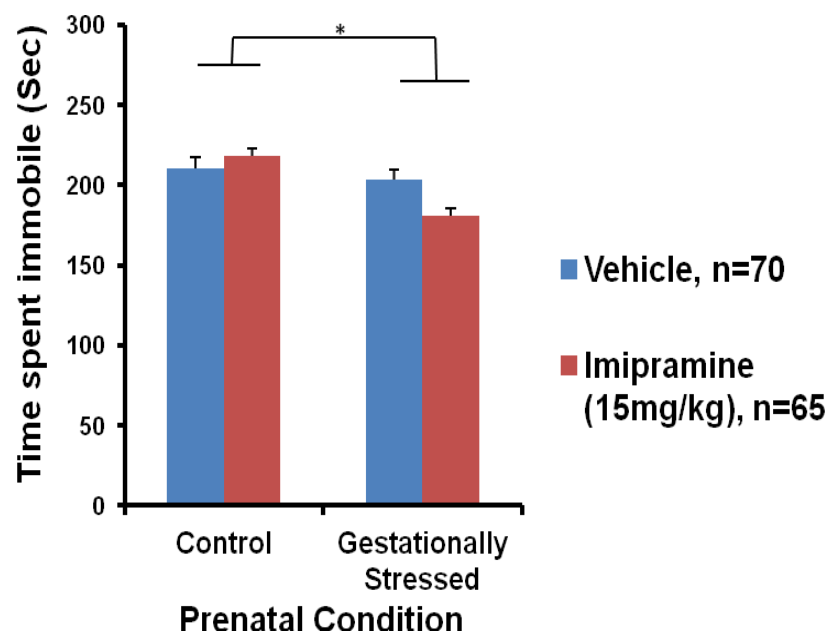


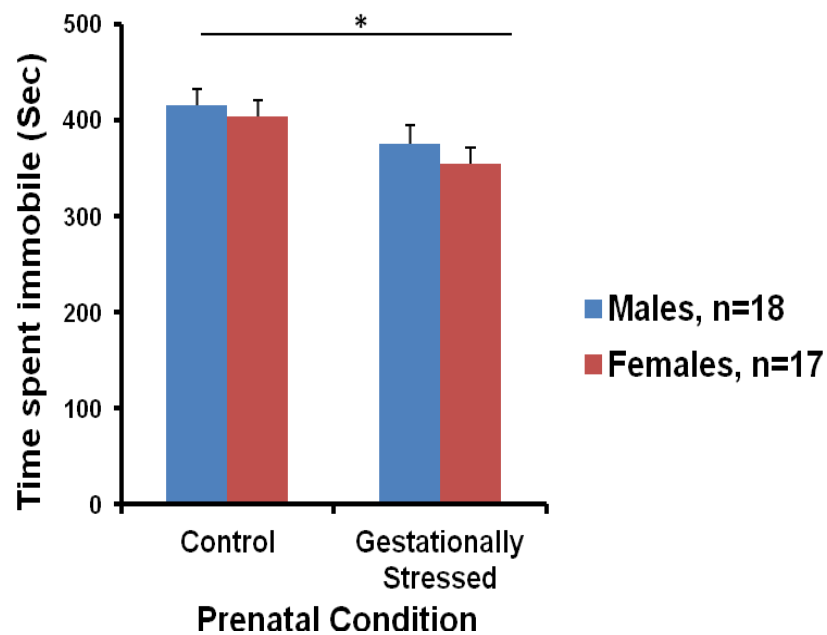
Figure 3-9: Offspring immobility times (from gestationally stressed or control Dams postnatally treated with Imipramine 15mg/kg or Vehicle (saline) after giving birth. The immobility of the offspring was assessed in two consecutive Exposure and Experimental Days when the offspring were 40-50 days old. Data shown are Means \pm SEM. *Significantly differences between offspring of control and gestationally stressed Dams and significant differences between vehicle and imipramine-treated animals, $p < 0.05$, Bonferroni t-test.

3.4.1 OFFSPRING PORSOLO AS STRESSED AND CONTROL

Figure 3-10 (Panel A) shows the immobility times for offspring of control and prenatally-stressed Dams and considering only gender and prenatal condition as orthogonal variables. In this instance, the postnatal drug treatment has been removed from the analysis for illustration purposes. An ANOVA on the data for the Exposure Day (Panel A) revealed a significant effect of prenatal condition on offspring immobility times with ($F(1,66)=6.71$, $p<0.012$), which means that females of prenatally-stressed Dams exhibited lower immobility times in comparison to males of prenatally stressed or Control Dams. There was no significant effect of gender immobility times with ($F(1,66)=0.9$, $p<ns$). Moreover, there was no significant effect of gender immobility times X prenatal condition interaction for these data with ($F(1,127)=0.07$, $p<ns$).

Figure 3-10 (Panel B) shows the immobility times for data obtained for the experimental day. An ANOVA on the data revealed no significant effect of prenatal condition on offspring immobility times with ($F(1,66)=0.62$, $p<ns$). There was no significant effect of gender immobility times with ($F(1,66)=2.72$, $p<ns$). Moreover, there was no significant effect of gender immobility times X prenatal condition interaction for these data with ($F(1,127)=0.002$, $p<ns$).

A. Exposure Day (10min)



B. Experimental Day (5min)

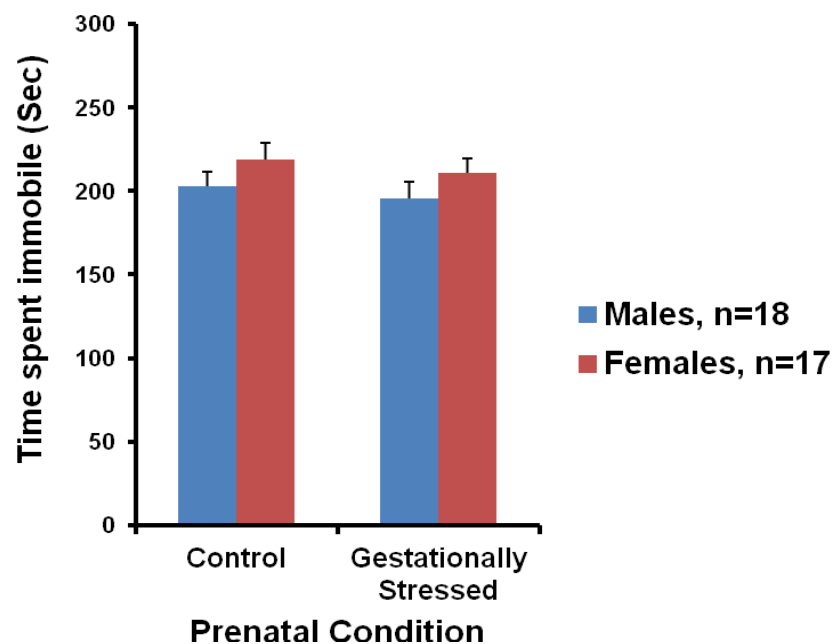


Figure 3-10: Immobility times for the Porsolt task recorded from the offspring (Males and female) of gestationally stressed or Control rat Dams on the Exposure and Experimental Days when offspring were 40-50 days old.

Data shown are Means \pm SEM.

*Significantly different, $p < 0.05$, Bonferroni t-test.

3.5 EFFECTS OF GESTATIONAL STRESS ON POSTNATAL ANXIETY IN DAMS

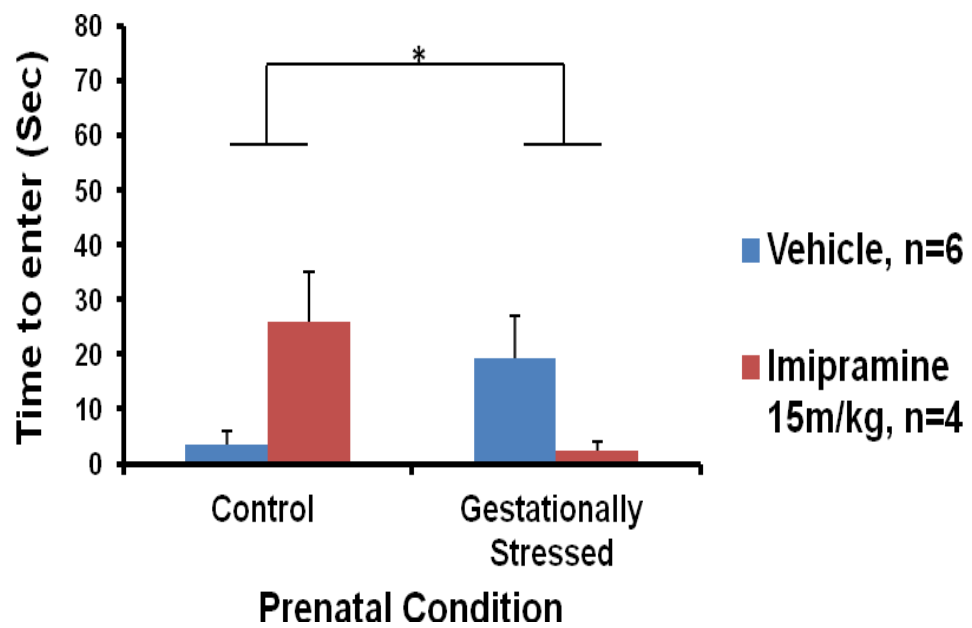
3.5.1 EFFECTS OF IMIPRAMINE

3.5.1.1 Time to enter

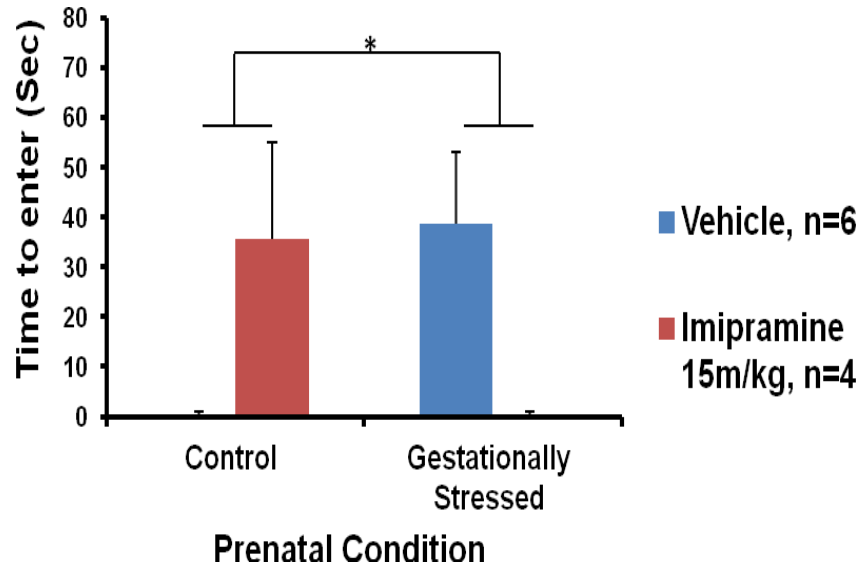
The Elevated Plus Maze (EPM) test was performed on groups of rats on postnatal days 4 and 10. The Dams were treated either with imipramine (15mg/kg) or Vehicle (saline) once daily following birth. Figure 3-11 shows the initial times for the Dams to enter the open arm (Days 4 and 10 - 5 minutes) trial. In Day 4 (Panel A), an ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=0.23$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on times to enter the open arms with ($F(1,6)=0.13$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed a significant effect with ($F(1,6)=5.82$, $p<0.05$). This shows that control Dams given imipramine postnatally spent more time in, in comparison with those treated with vehicle and vice versa happened in stressed Dams.

In Day 10 (Panel B), an ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=0.01$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on times to enter the open arms with ($F(1,6)=0.01$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed a significant effect with ($F(1,6)=5.7$, $p<0.05$), which shows that control Dams were given imipramine postnatally spent more time in comparison with those treated with vehicle and vice versa happened in stressed Dams.

A. Open arm (Day 4)



B. Open arm (Day 10)



3-11: Effects of gestational stress and postnatal drug treatment on initial times to enter the open arms on days 4 and 10 of the elevated plus maze test.

Data shown are Means \pm SEM.

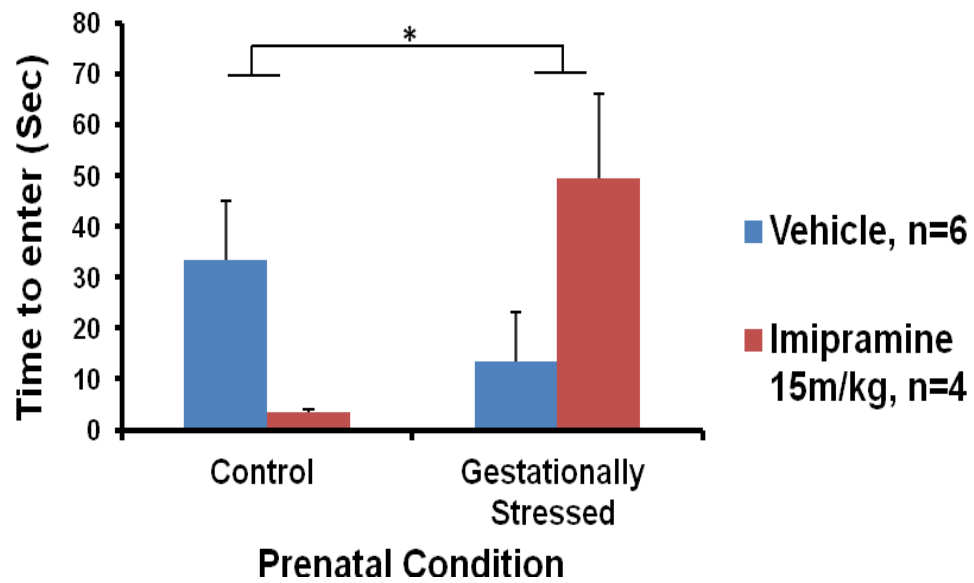
*Significantly different, $p < 0.05$, Bonferroni t-test.

Figure 3-12 shows the initial times for the Dams to enter the closed arms (Days 4 and 10-5 minutes) trial.

In Day 4 (Panel A), an ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=1.22$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on times to enter the closed arms with ($F(1,6)=0.07$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed a significant effect with ($F(1,6)=7.83$, $p<0.03$), which shows that control Dams given the vehicle postnatally spent more time in, in comparison with those treated with imipramine, however, vice versa happened in stressed Dams.

In Day 10 (Panel B), an ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=0.05$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on times to enter the closed arms with ($F(1,6)=0.42$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=0.03$, $p<ns$).

A. Closed arm (Day 4)



B. Closed arm (Day 10)

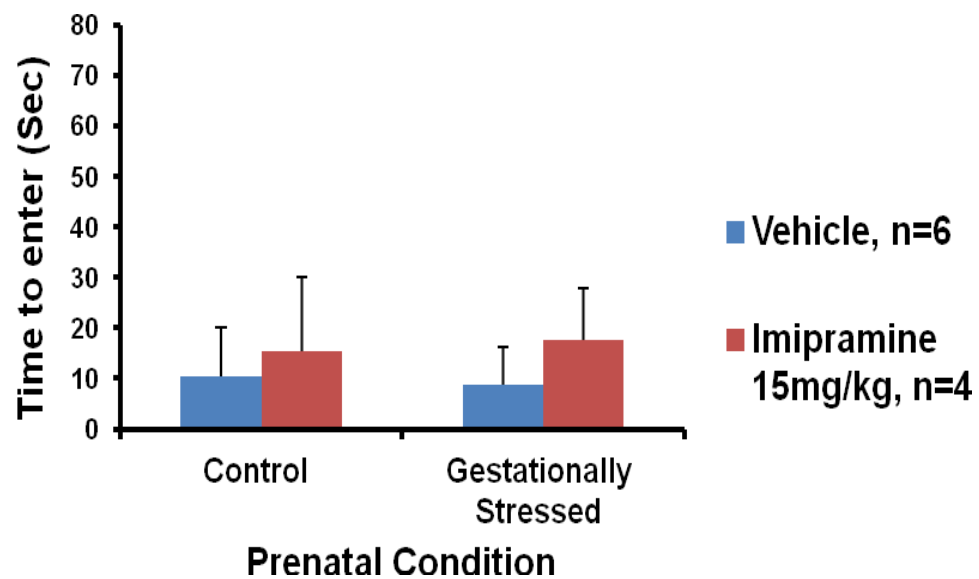


Figure 3-12: Effects of gestational stress and postnatal drug treatment on initial times to enter the closed arms on days 4 and 10 of the elevated plus maze test.

Data shown are Means \pm SEM.

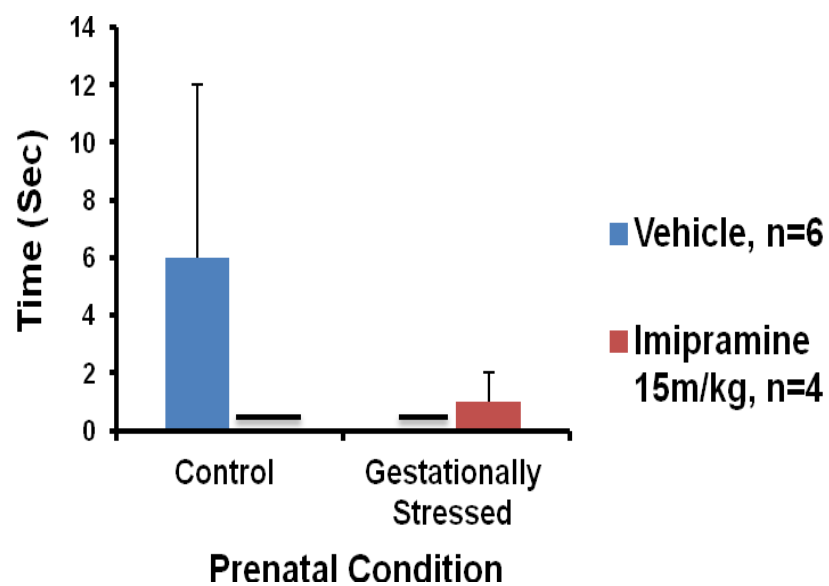
*Significantly different, $p < 0.05$, Bonferroni t-test.

3.5.1.2 Licking and Grooming

Figure 3-13 shows the amount of time the Dams spent licking and grooming in the open arms. In day 4 (Panel A), an ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=1.16$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on L/G with ($F(1,6)=1.16$, $p<ns$). An ANOVA examining the prenatal condition by postnatal drug interaction revealed no significant effect with ($F(1,6)=2.27$, $p<ns$).

Figure 3-13 (Panel B) shows the amount of time for the Dams spent licking and grooming in the open arms day 10. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=1.71$, $p<ns$). Furthermore there was no significant effect of postnatal drug treatment on L/G in the open arms with ($F(1,6)=1.71$, $p<ns$). An ANOVA examining the prenatal condition by postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=1.71$, $p<ns$).

A. Open arm (Day 4)



B. Open arm (Day 10)

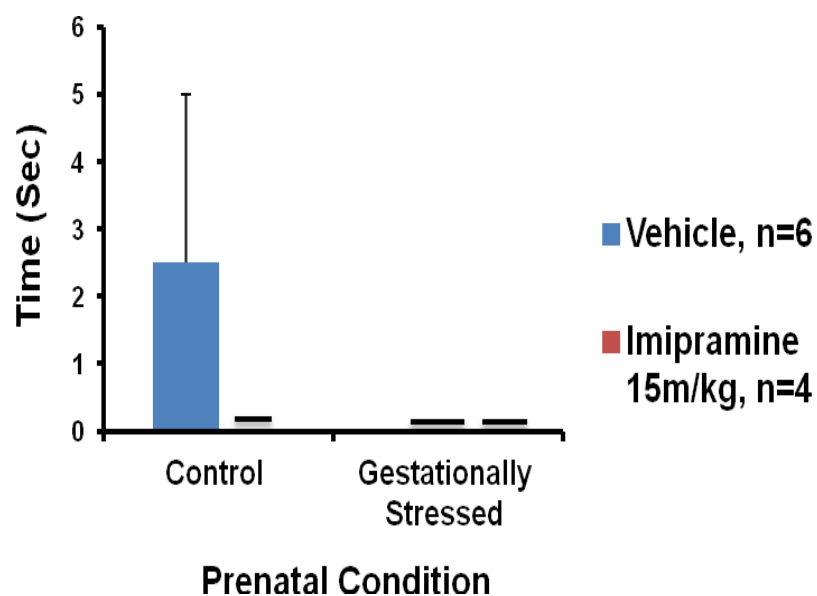
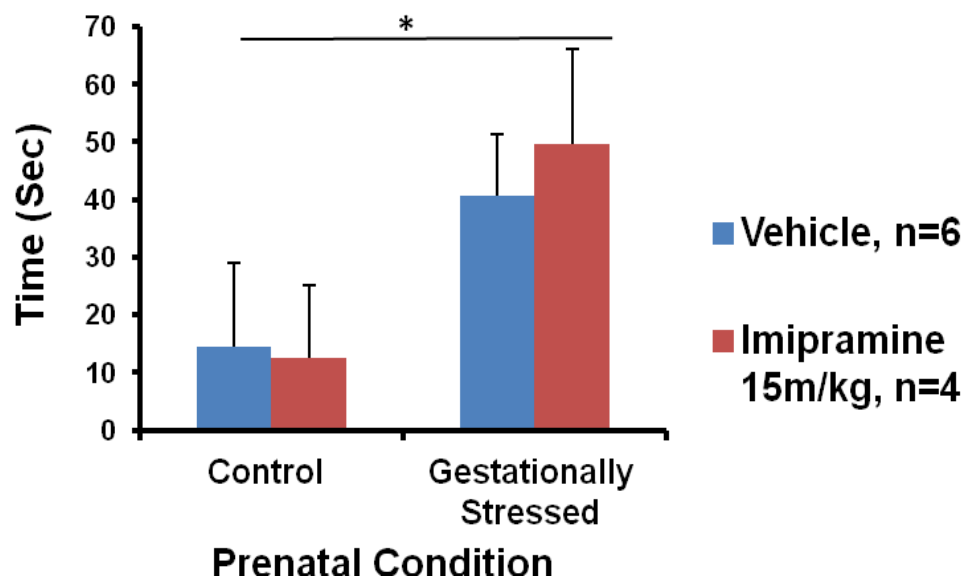


Figure 3-13: Effects of gestational stress and postnatal drug treatment on time spent grooming in the open arms for Dams tested in the elevated plus maze on postnatal days 4 and 10. Data shown are Means \pm SEM.

Figure 3-14 shows the amount of time the Dams spent licking and grooming in the closed arms. In day 4 (Panel A), an ANOVA on these data revealed a significant effect of prenatal condition with ($F(1,6)=5.24$, $p<0.05$), which shows stressed Dams spent more time grooming in the closed arms in comparison with control Dams. Furthermore, there was no significant effect of postnatal drug treatment on L/G with ($F(1,6)=0.06$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=0.15$, $p<ns$).

Figure 3-14 (Panel B) shows the amount of time for the Dams spent licking and grooming in the closed arms day 10. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=1.58$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on L/G at the closed arms with ($F(1,6)=2.05$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=0.24$, $p<ns$).

A. Closed arm (Day 4)



B. Closed arm (Day 10)

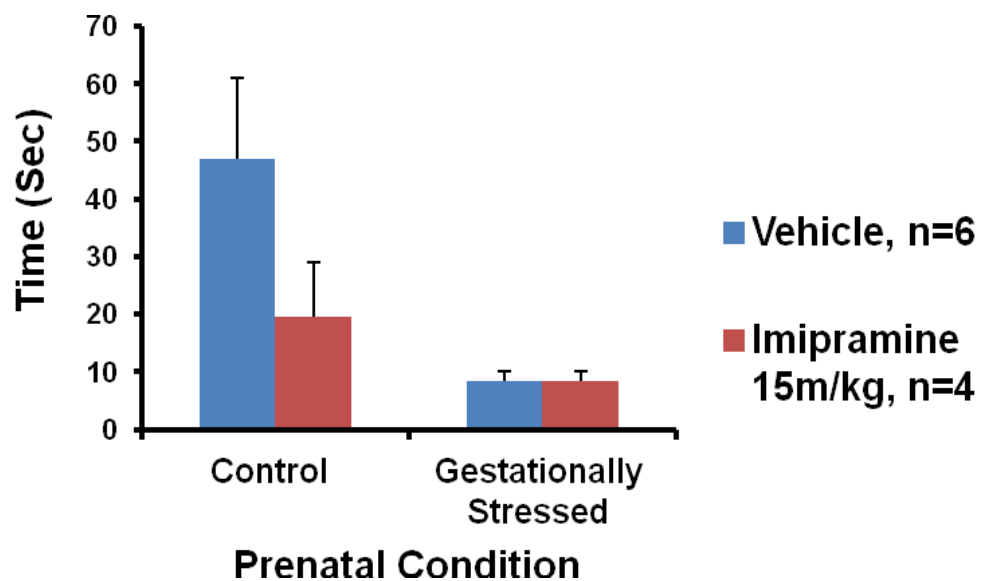


Figure 3-14: Effects of gestational stress and postnatal drug treatment on time spent grooming in the closed arms for Dams tested in the elevated plus maze on postnatal days 4 and 10. Data shown are Means \pm SEM.

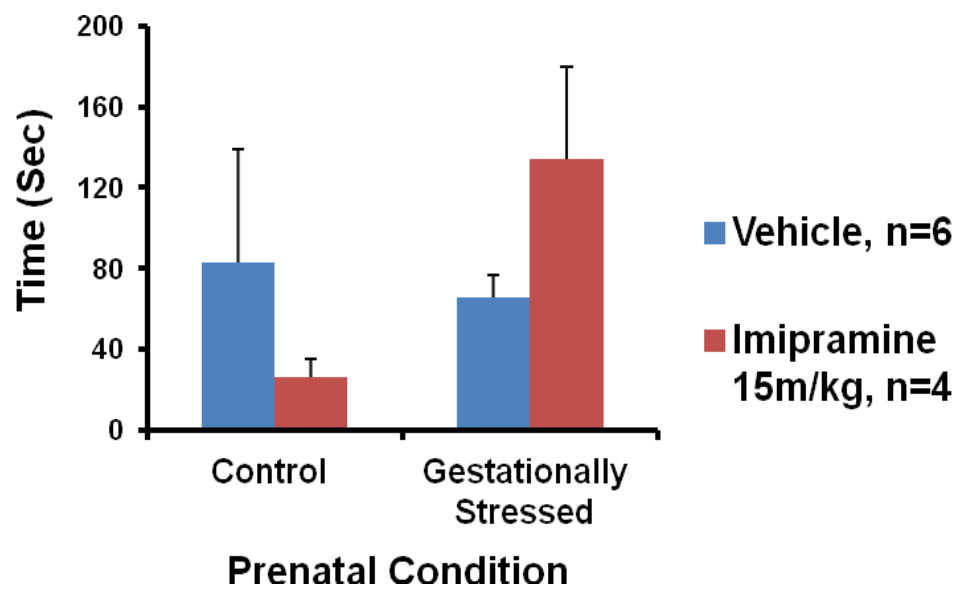
*Significantly different, $p < 0.05$, Bonferroni t-test.

3.5.1.3 Total Time

Figure 3-15 (Panel A) shows the total time that Dams spent in the open arms Day 4. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=2.33$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on total time with ($F(1,6)=0.04$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=4.43$, $p<ns$).

Figure 3-15 (Panel B) shows the total time that Dams spent in the open arms Day 10. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=0.05$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on total time with ($F(1,6)=0.19$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=0.48$, $p<ns$).

A. Open arm (Day 4)



B. Open arm (Day 10)

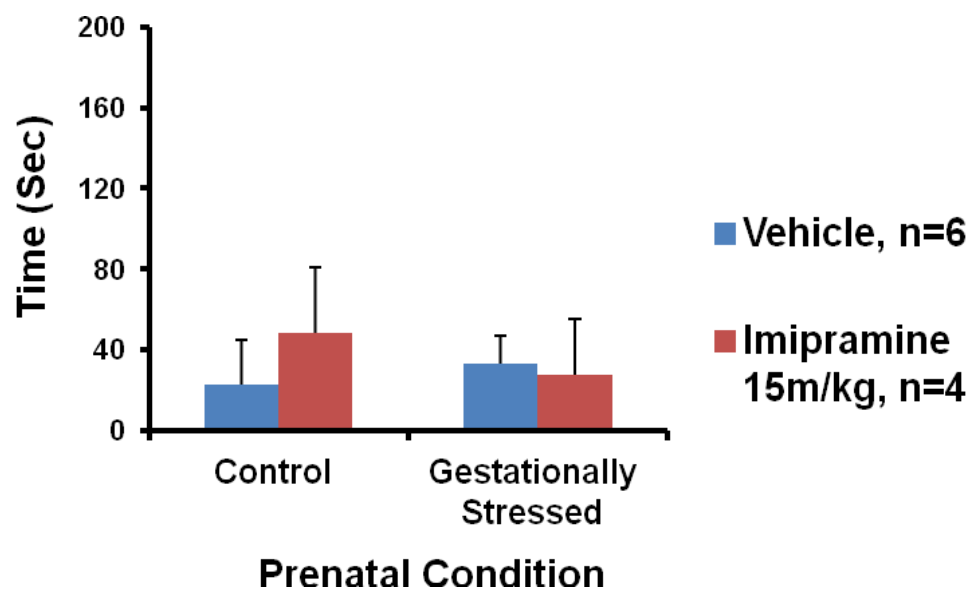
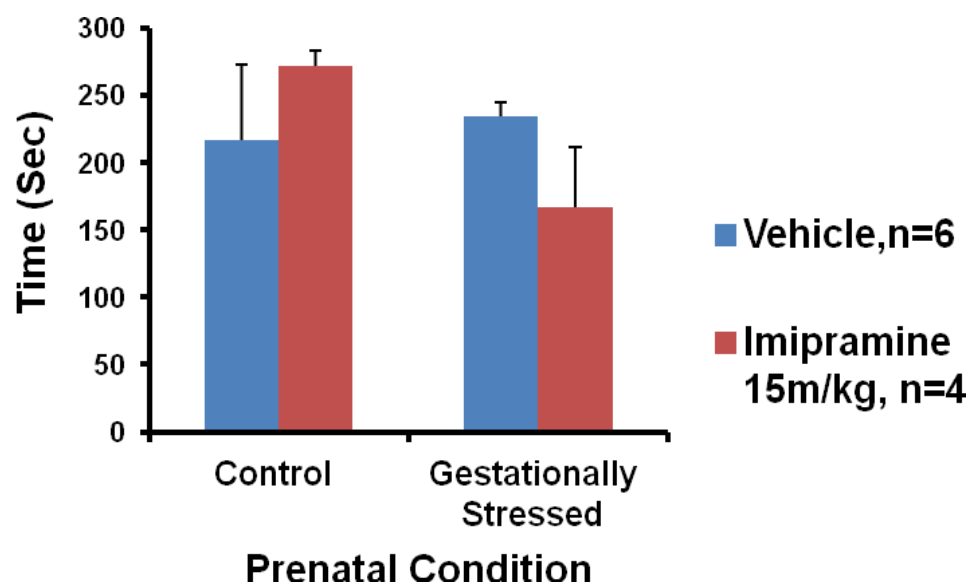


Figure 3-15: Effects of gestational stress and postnatal drug treatment on total time in the open arms the elevated plus maze for Dams tested on postnatal days 4 and 10. Data shown are Means \pm SEM.

Figure 3-16 (Panel A) shows the total time that Dams spent in the closed arms on Day 4. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=2.20$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on total time with ($F(1,6)=0.05$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=4.3$, $p<ns$).

Figure 3-16 (Panel B) shows the total time for that Dam spent in the closed arms Day 10. An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,6)=0.05$, $p<ns$). Furthermore, there was no significant effect of postnatal drug treatment on total time with ($F(1,6)=0.19$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,6)=0.5$, $p<ns$).

A. Closed arm (Day 4)



B. Closed arm (Day 10)

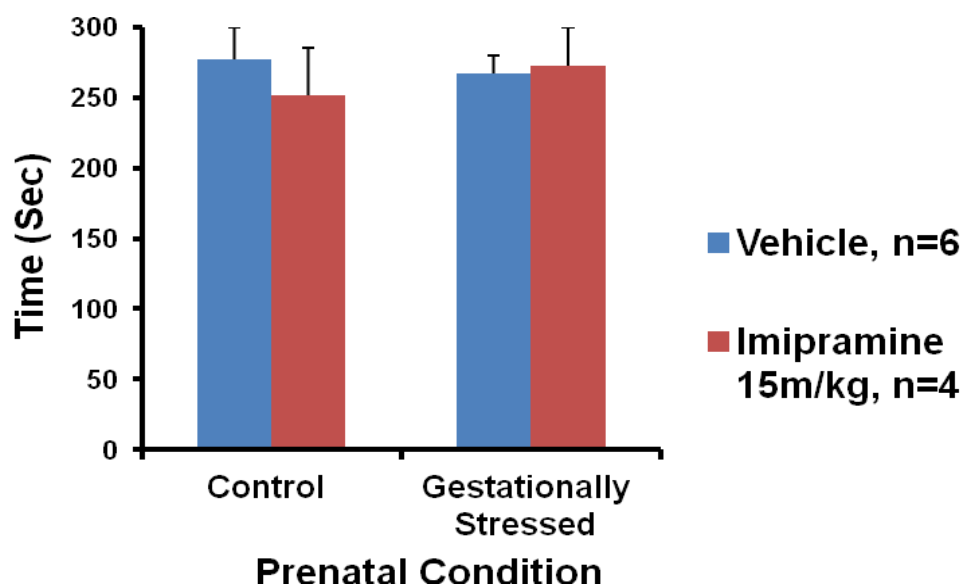


Figure 3-16: Effects of gestational stress and postnatal drug treatment on total time in the closed arms the elevated plus maze for Dams tested on postnatal days 4 and 10. Data shown are Means \pm SEM.

3.5.2 OFFSPRING ELEVATED PLUS MAZE AND THE EFFECTS OF SERTRALINE

3.5.2.1 Time to enter

a) Open arms

Figure 3-17 (Panel A) shows the initial times for the offspring to enter the open and closed arms for EPM. This test lasted for 5 minutes.

Each group was randomly selected from Dams who had undergone restraint stress or were unstressed controls and had been treated with either sertraline (10mg/kg) or vehicle (saline) postnatally from day 1-10.

An ANOVA on the data for times to enter the open arms revealed no significant effect of prenatal condition with ($F(1,192)=0.1$, $p<ns$). An ANOVA on the data for times to enter the open arms revealed no significant effect of postnatal drug treatment with ($F(1,192)=0.4$, $P<ns$). There was no significant effect of gender times to enter open arms with ($F(1,192)=0.03$, $P<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,192)=0.01$, $p<ns$). An ANOVA examining the prenatal condition X gender times to enter interaction revealed no significant effect with ($F(1,192)=1.2$, $p<ns$). An ANOVA examining postnatal drug treatment X gender interaction revealed no significant effect with ($F(1,192)=0.2$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender times to enter in the open arms interaction revealed no significant effect with ($F(1,192)=0.6$, $p<ns$).

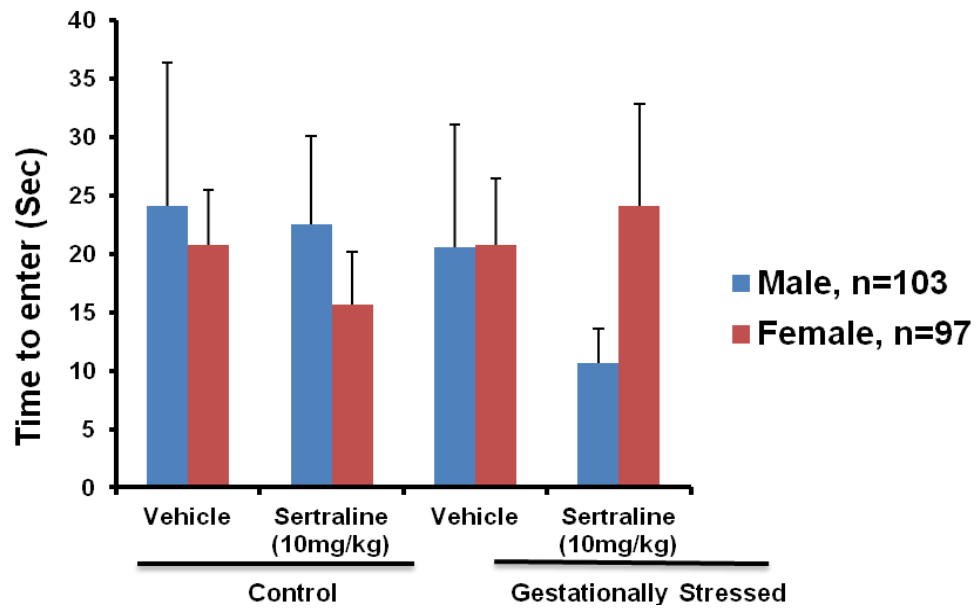
b) Closed arms

Figure 3-17 (Panel B) shows the initial times for the offspring to enter the closed arms for EPM.

Each group was randomly selected from Dams who had undergone restraint stress or were unstressed controls and had been treated with either sertraline (10mg/kg) or vehicle (saline) postnatally from day 1-10.

An ANOVA on the data for times to enter the closed arms revealed no significant effect of prenatal condition with ($F(1,192)=0.01$, $p<ns$). An ANOVA on the data for times to enter the closed arms revealed no significant effect of postnatal drug treatment with ($F(1,192)=0.33$, $P<ns$). There was no significant effect of gender times to enter the closed arms with ($F(1,192)=0.01$, $P<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,192)=3.34$, $p<ns$). An ANOVA examining the prenatal condition X gender times to enter the closed arms interaction revealed no significant effect with ($F(1,192)=0.6$, $p<ns$). An ANOVA examining postnatal drug treatment X gender times to enter the closed arms interaction revealed no significant effect with ($F(1,192)=0.4$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender times to enter the closed arms interaction revealed no significant effect with ($F(1,192)=0.04$, $p<ns$).

A. Open arm



B. Closed arm

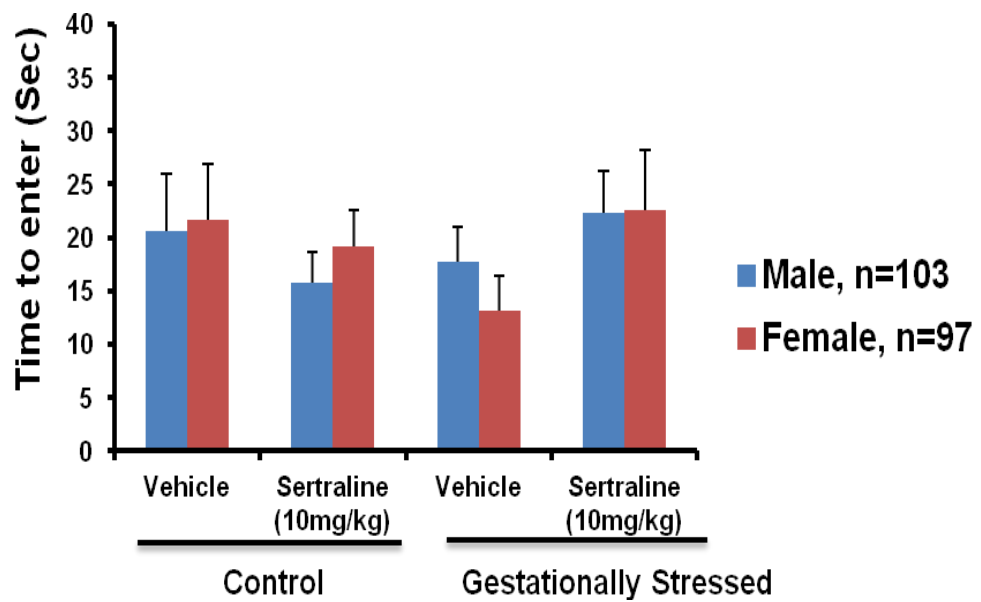


Figure 3-17: Shows offspring time to enter the open and closed arms. Behaviour of offspring during the elevated plus maze (EPM). These were born of Dams which had been left undisturbed during gestation (Control), or who had been subjected to gestational stress. At birth Dams were treated with once daily injections of saline or sertraline (10mg/kg) up to and including postnatal day 10. Data shown are Means \pm SEM.

3.5.2.2 Licking and Grooming

Figure 3-18 (Panel A) shows the amount of times the offspring spent licking and grooming in the open arms.

a) Open arms

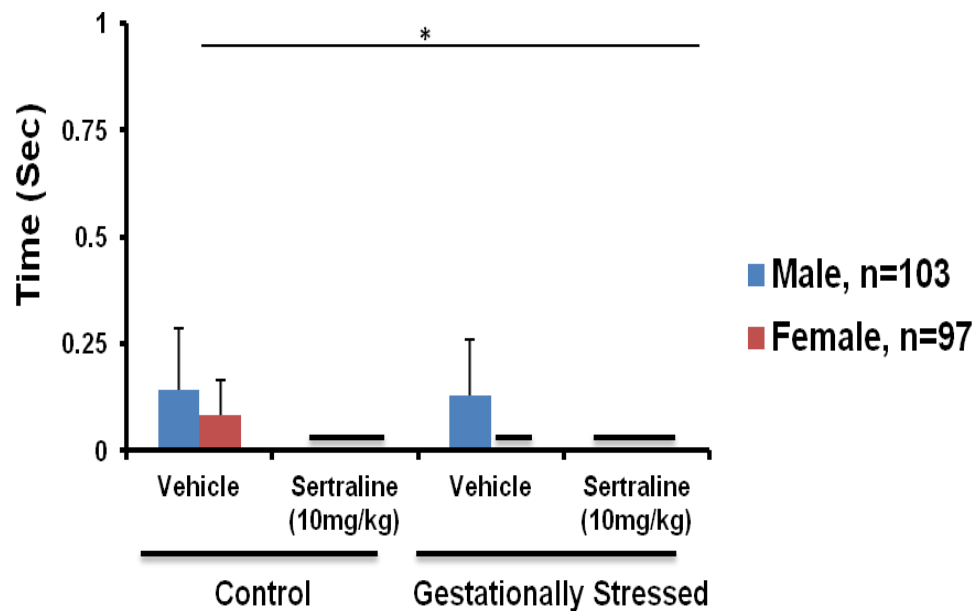
An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,192)=0.25$, $p<ns$). An ANOVA on these data revealed a significant effect of postnatal drug treatment with ($F(1,192)=3.5$, $p<0.05$), showing that offspring of Dams either control or prenatally-stressed which had been treated with sertraline had less time spent grooming in comparison with those which had been treated with vehicle. Furthermore, There was no significant effect of gender with ($F(1,192)=1$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,192)=0.25$, $p<ns$). ANOVA examining the prenatal condition X gender L/G in the open arms revealed no significant effect interaction with ($F(1,192)=0.14$, $p<ns$). Moreover, an ANOVA examining the drug treatment X gender L/G in the open arms interaction revealed no significant effect with ($F(1,192)=1$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender L/G in the open arms interaction revealed no significant effect with ($F(1,192)=0.14$, $p<ns$).

b) Closed arms

Figure 3-18 (Panel B) shows the amount of times the offspring spent licking and grooming in the closed arms.

An ANOVA on these data revealed a significant effect of prenatal condition with ($F(1,192)=3.6$, $p<0.05$), which shows overall the control Dams spent more times licking and grooming in comparison with prenatally-stressed Dams. An ANOVA on these data revealed no significant effect of postnatal drug treatment with ($F(1,192)=0.001$, $p<ns$). There was no significant effect of gender L/G in the closed arms with ($F(1,192)=1.4$, $p<ns$). Furthermore, an ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed a significant effect with ($F(1,192)=5.6$, $p<0.02$), which shows offspring from control Dams that were given vehicle spent more times licking and grooming in comparison with those of offspring from stressed Dams that were given sertraline postnatally. Moreover, An ANOVA examining the prenatal condition X gender L/G in the closed arms interaction revealed no significant effect with ($F(1,192)=2.75$, $p<ns$). An ANOVA examining the drug treatment X gender L/G in the closed arms interaction revealed no significant effect with ($F(1,192)=0.15$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender L/G in the closed arms interaction revealed no significant effect with ($F(1,192)=0.45$, $p<ns$).

A. Open arm



B. Closed arm

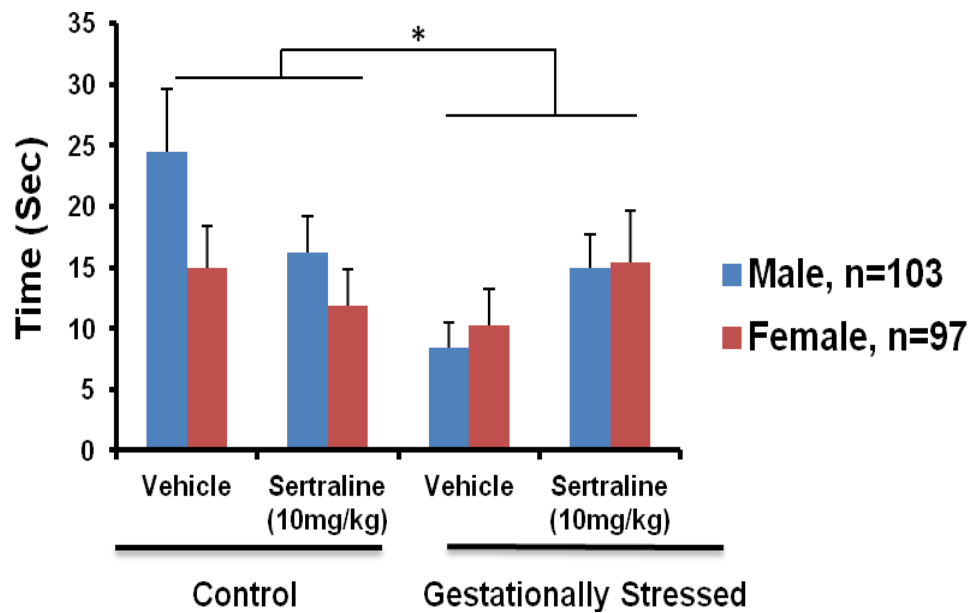


Figure 3-18: Shows offspring time spent grooming in the open and closed arms. These were born of Dams which had been left undisturbed during gestation (Control), or who had been subjected to gestational stress. At birth Dams were treated with once daily injections of saline or sertraline (10mg/kg) up to and including postnatal day 10. a) Times spent in open and closed arms

3.5.2.3 Total Times spent in open and closed arms

Figure 3-19 (Panel A) shows the total time that offspring spent in open or closed arms.

a) Open arms

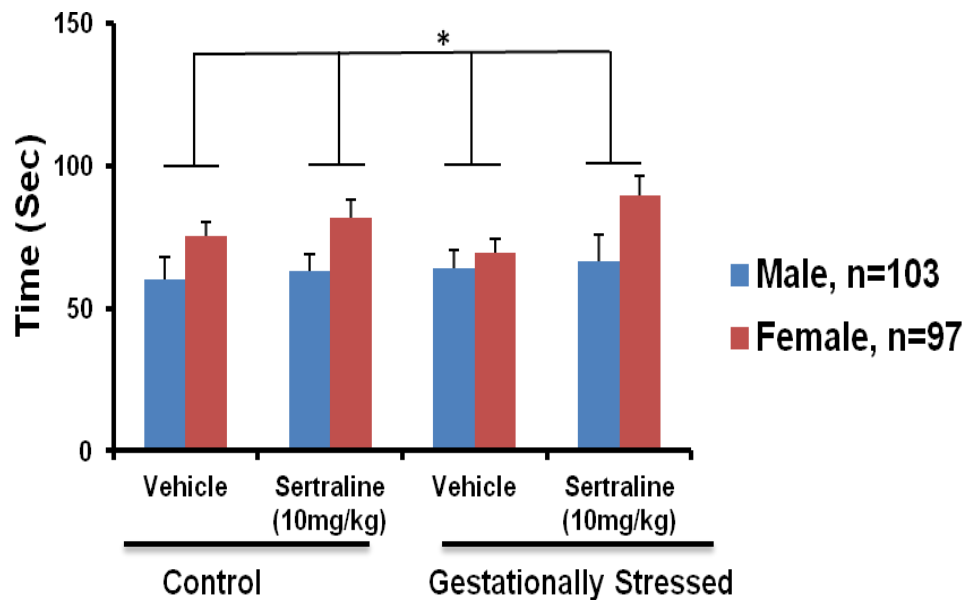
An ANOVA on these data revealed no significant effect of prenatal condition with ($F(1,192)=0.25$, $p<ns$). An ANOVA on these data revealed no significant effect of postnatal drug treatment with ($F(1,192)=2.83$, $p<ns$). Furthermore, there was a significant effect of gender with ($F(1,192)=10.743$, $p<0.001$), which showed the total time to spend for females was higher than males. An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed no significant effect with ($F(1,192)=0.46$, $p<ns$). Moreover, an ANOVA examining the prenatal condition X gender total times in the open arms interaction revealed no significant effect with ($F(1,192)=0.11$, $p<ns$). An ANOVA examining the drug treatment X gender total times in the open arms interaction revealed no significant effect with ($F(1,192)=1.18$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender total times in the open arms interaction revealed no significant effect with ($F(1,192)=0.55$, $p<ns$).

a) Closed arms

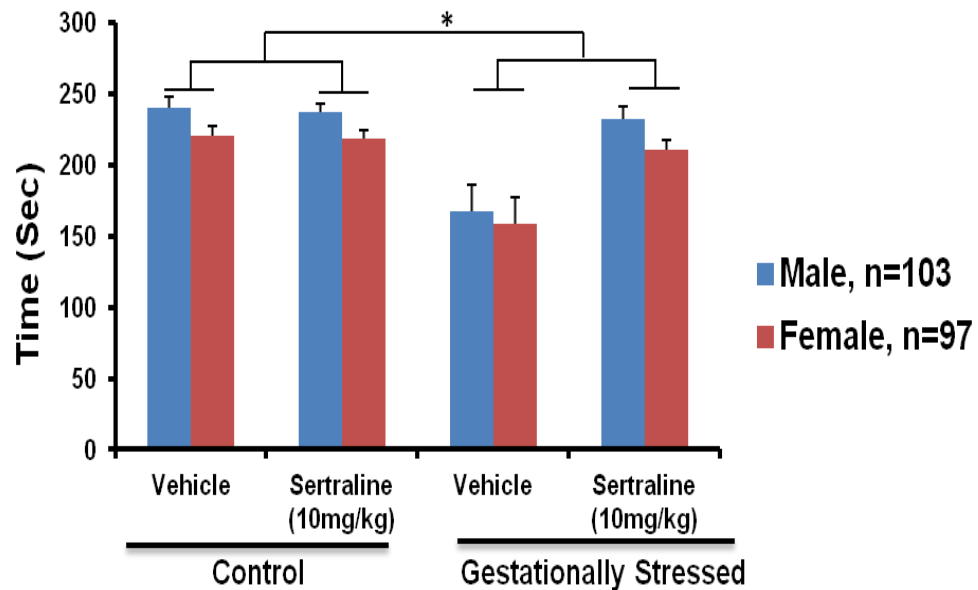
Figure 3-19 (Panel B) shows the amount of time that offspring spent in the closed arms.

An ANOVA on these data revealed a significant effect of prenatal condition with ($F(1,192)=24.32$, $p<0.0001$), which showed that offspring of control Dams exhibited more amount of time to spend in the closed arms in comparison to those from prenatally-stressed Dams. An ANOVA on these data revealed a significant effect of postnatal drug treatment with ($F(1,192)=14.52$, $p<0.0002$), showing that offspring of Dams treated with sertraline spent more amount of time in the closed arms in comparison to those treated with vehicle. Furthermore, there was a significant effect of total times on gender in the closed arms with ($F(1,192)=5.4$, $p<0.02$), which showed females spent less total amount of time in the closed arms in comparison with males. An ANOVA examining the prenatal condition X postnatal drug treatment interaction revealed a significant effect with ($F(1,192)=17.15$, $p<0.0001$), which showed offspring of control Dams were either given vehicle or sertraline spent more amount of total time times in comparison with those offspring of prenatally-stressed Dams. An ANOVA examining the prenatal condition X gender total times in the closed arms interaction revealed no significant effect with ($F(1,192)=0.07$, $p<ns$). Moreover, an ANOVA examining the drug treatment X gender interaction revealed no significant effect with ($F(1,192)=0.17$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X gender total times in the closed arms interaction revealed no significant effect with ($F(1,192)=0.2$, $p<ns$).

A. Open arm



B. Closed arm



3-19: Shows offspring amount of total time spent in the open and closed arms. Behaviour of offspring during the elevated plus maze (EPM). These were born of Dams which had been left undisturbed during gestation (Control), or who had been subjected to gestational stress. At birth Dams were treated with once daily injections of saline or sertraline (10mg/kg) up to and including postnatal day 10. Data shown are Means \pm SEM.

*Significantly different, $p < 0.05$, Bonferroni t-test.

3.6 MATERNAL BEHAVIOUR: EFFECTS OF GESTATIONAL STRESS AND TREATMENT WITH IMIPRAMINE

3.6.1 ARCHED BACK NURSING (ABN)

Maternal behavioural assessments were made on groups of rats over postnatal days 3, 5 and 7. The Dams had been treated either with imipramine (15mg/kg) or vehicle (saline) once daily following birth. Figure 3-20 shows the amount of time the Dams spent in Arched-Back Nursing (ABN) position. An ANOVA on the data revealed a significant effect of prenatal condition with ($F(1,32)=5.7$, $p<0.03$), showing that stressed Dams spent less time in ABN compared to control Dams. ANOVA revealed no significant effect of postnatal drug treatment on time ABN with ($F(1,32)=1.3$, $p<ns$). An ANOVA examining the interaction between postnatal drug treatment and prenatal condition revealed no significant effect with ($F(1,32)=1.27$, $p<ns$). ANOVA revealed no significant change in ABN over postnatal days 3, 5 and 7 with ($F(1,32)=2.27$, $p<ns$). Furthermore, an ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.3$, $p<ns$). An ANOVA examining the postnatal drug treatment X postnatal days interaction revealed no significant effect with ($F(1,32)=2.04$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=2.07$, $p<ns$).

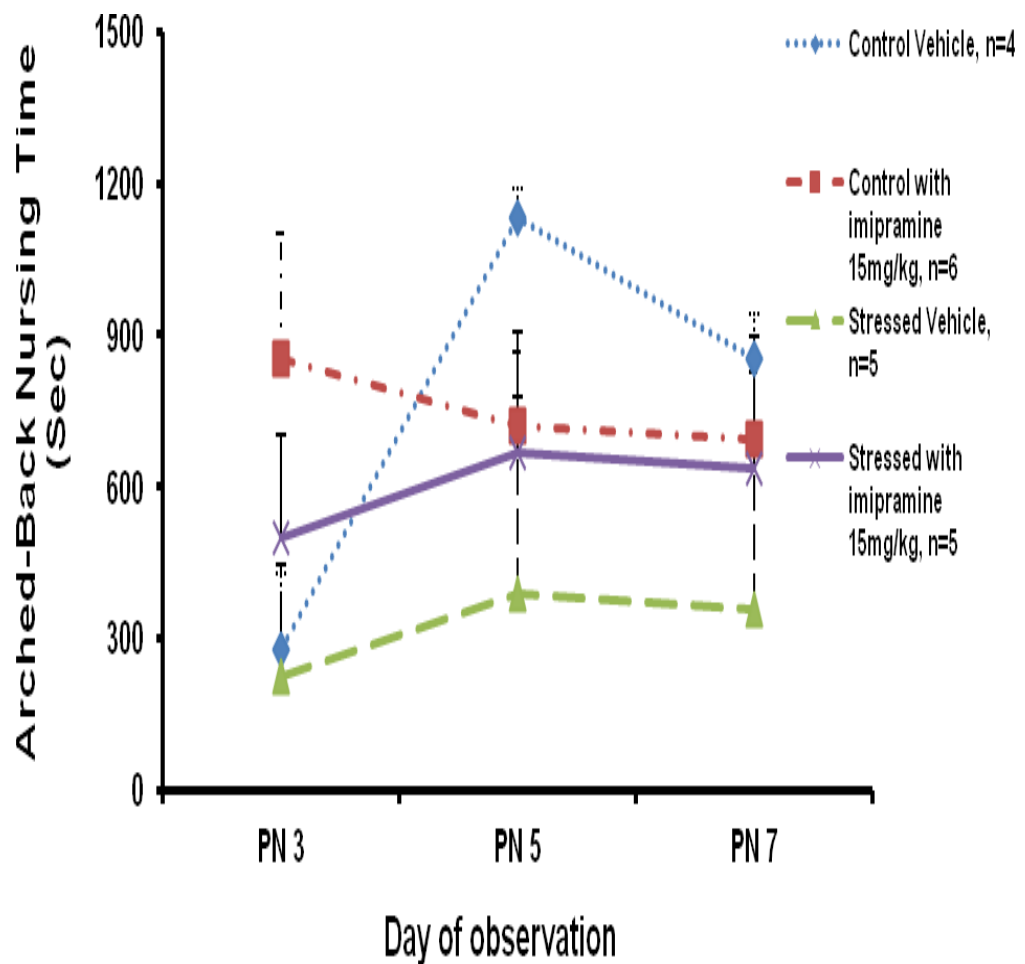


Figure 3-20: Arched-Back nursing times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.
Data shown are Means \pm SEM.

3.6.2 SUPINE NURSING

Figure 3-21 shows the amount of time that Dams spent in a supine nursing position. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=0.35$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on time spent in a supine nursing position with ($F(1,32)=0.64$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.145$, $p<ns$). However, ANOVA on these data did show a significant effect of testing day (3, 5 and 7) on supine nursing times with ($F(1,32)=4.5$, $p<0.02$) and this shows that all Dams spent more time in a supine nursing position as the infants aged. An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.332$, $p<ns$). An ANOVA examining the postnatal drug treatment X postnatal days interaction revealed no significant effect with ($F(1,32)=0.62$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.31$, $p<ns$).

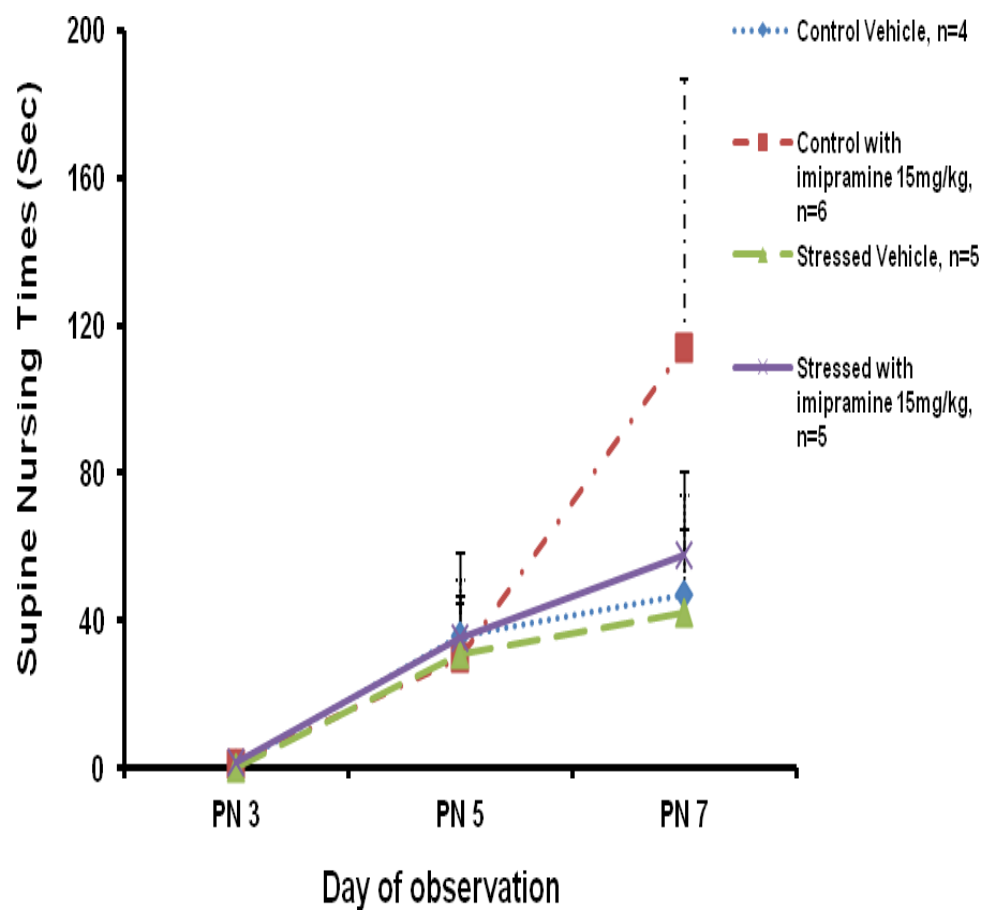


Figure 3-21: Supine nursing times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

3.6.3 LICKING/GROOMING PUPS (LG)

Figure 3-22 shows the amount of time that Dams spent Licking and Grooming (LG) their pups. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=2.51$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on L/G with ($F(1,32)=0.28$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.21$, $p<ns$). ANOVA did reveal a significant effect of testing day (3, 5 and 7) on Dams L/G their pups with ($F(1,32)=10.9$, $p<0.0002$) and this shows that all Dams spent more time L/G their pups as the litters get older. An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.01$, $p<ns$). An ANOVA examining the postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=1.8$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.04$, $p<ns$).

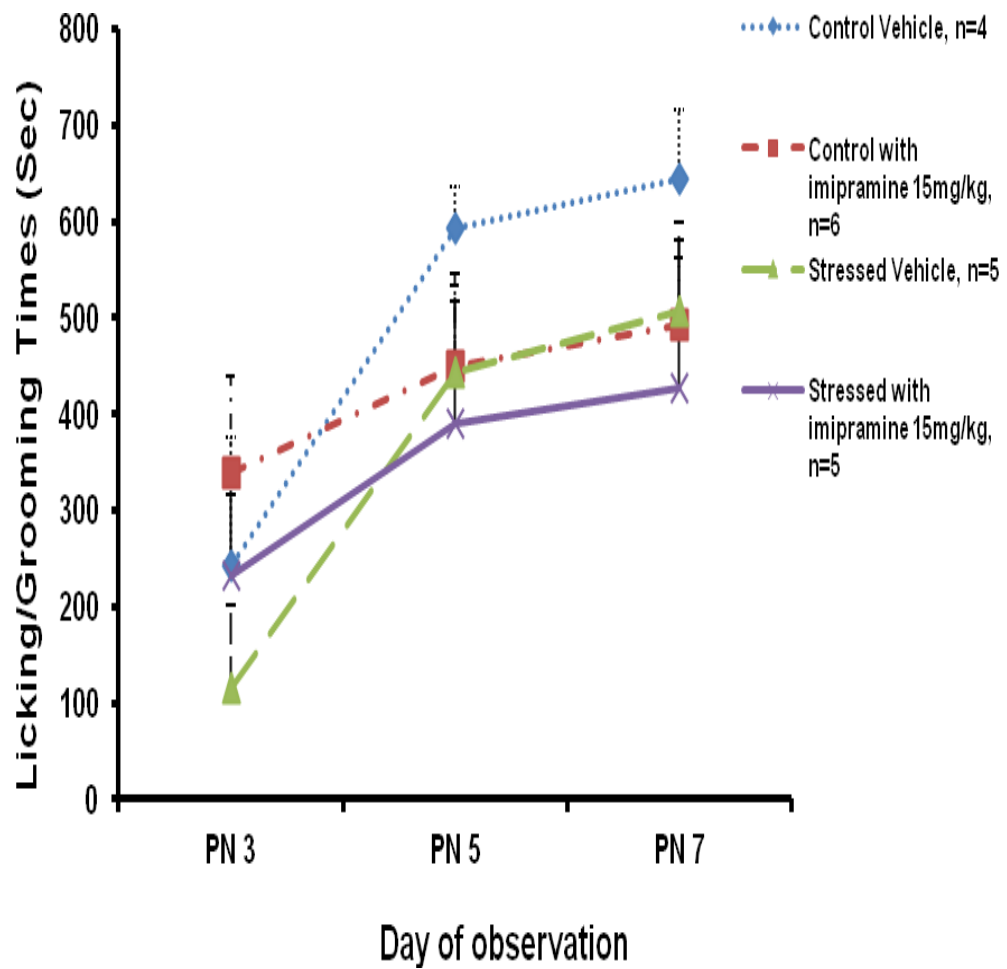


Figure 3-22: Licking/Grooming times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

3.6.4 PUP RETRIEVAL

Figure 3-23 shows the amount of time the Dams spent engaged in retrieving their pups. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=2.38$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on pup retrieval with ($F(1,32)=1.58$, $p<ns$). An ANOVA examining the interaction between postnatal drug treatment and prenatal condition revealed no significant effect with ($F(1,32)=0.4$, $p<ns$). ANOVA revealed no significant effect on pup retrieval over postnatal days (3, 5 and 7) with ($F(1,32)=0.314$, $p<ns$). An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=2.05$, $p<ns$). An ANOVA examining the postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=4.14$, $p<0.02$) and this shows that Dams treated with imipramine spent more time retrieving pups in comparison with Dams administered vehicle. An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=4.25$, $p<0.02$), which shows that stressed Dams administered imipramine exhibited markedly enhanced pup retrieving on postnatal days 5 and 7.

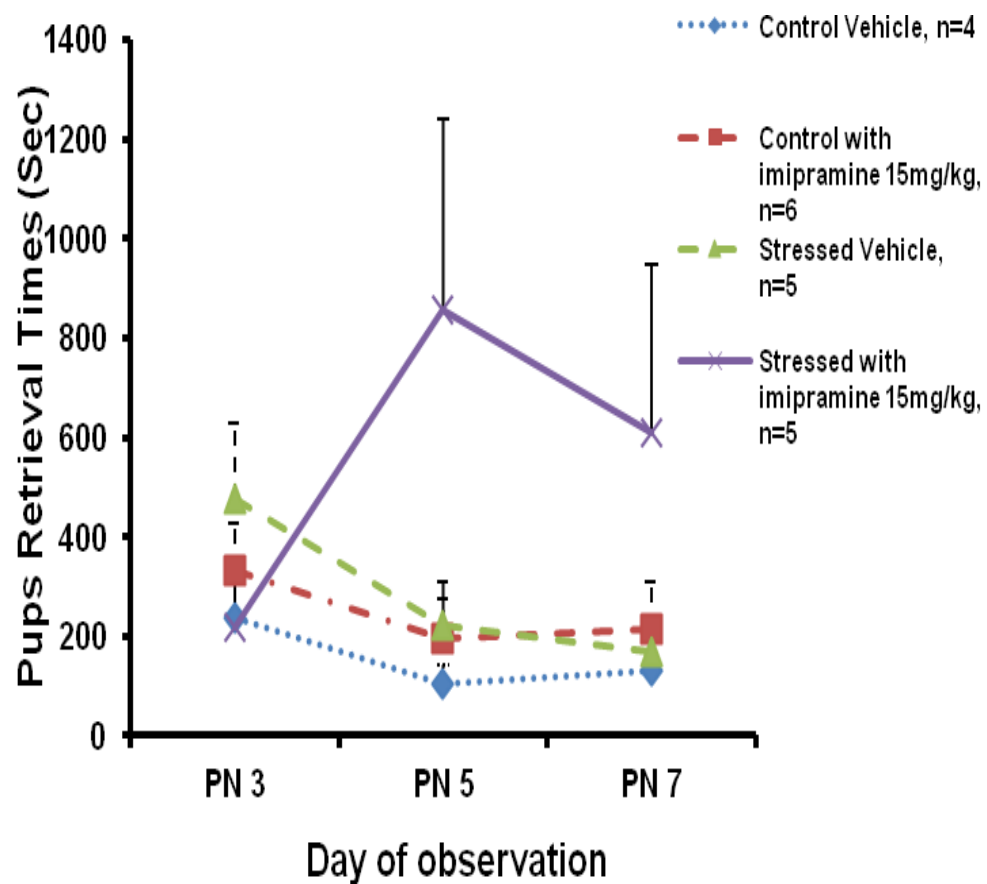


Figure 3-23: Time spent by Dams engaged in actual pup retrieval during the 30 minute observation period.
Data shown are Means \pm SEM.

3.6.5 TIME SPENT IN THE NEST

Figure 3-24 shows the amount of time the Dams spent in the Nest. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=2.54$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on time spent in the nest with ($F(1,32)=0.002$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.08$, $p<ns$). ANOVA did reveal a significant effect of test day (3, 5 and 7) on Dams time spent in the nest with ($F(1,32)=6.28$, $p<0.005$) and this shows that all Dams spent more time in the nest as the litters get older. An ANOVA examining the prenatal condition X postnatal days interaction revealed no significant effect with ($F(1,32)=0.052$, $p<ns$). An ANOVA examining the postnatal drug treatment X postnatal days interaction revealed a significant effect with ($F(1,32)=2.8$, $p<0.05$), which shows that stressed Dams treated with imipramine spent less time in the nest compared to control Dams and this time enhanced on postnatal days 5 and 7. An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.32$, $p<ns$).

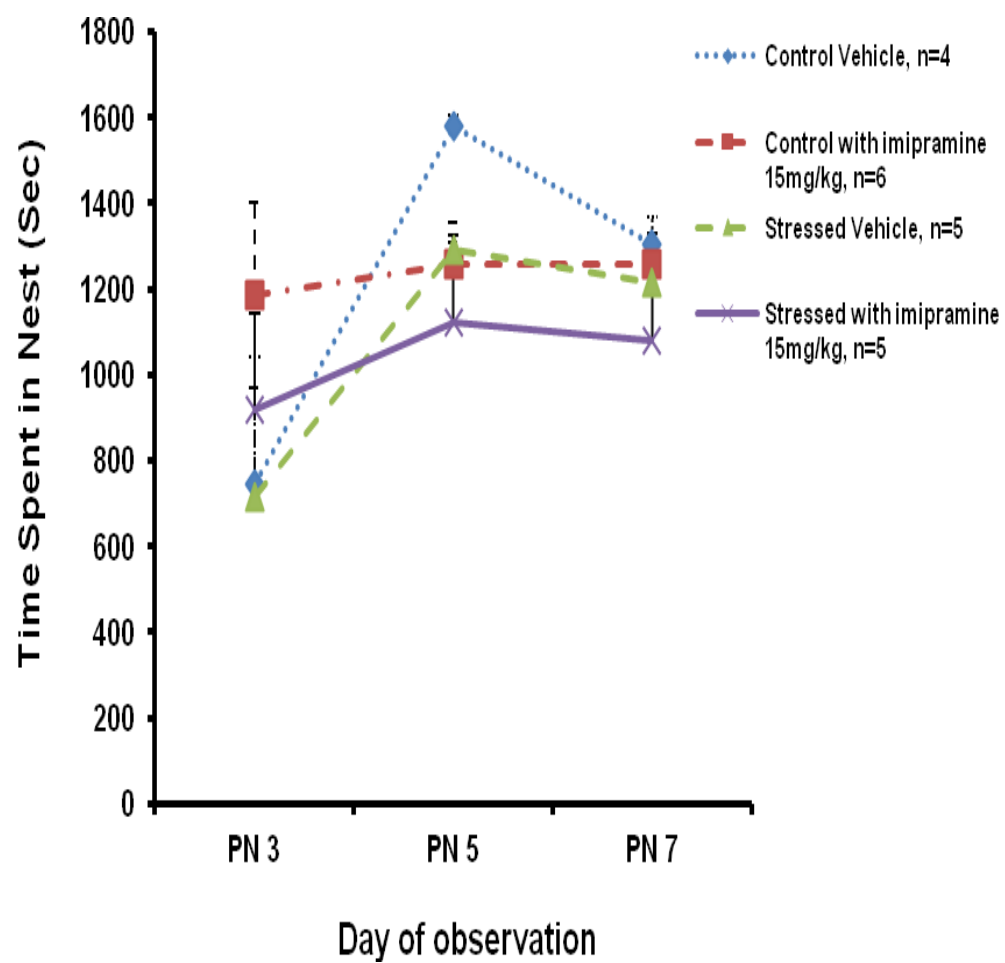


Figure 3-24: Time spent in Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

3.6.6 TIME SPENT OUT OF THE NEST

Figure 3-25 shows the amount of time the Dams spent out of the nest. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=2.70$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on time spent out of the nest with ($F(1,32)=0.01$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.104$, $p<ns$). ANOVA revealed a significant effect of test day (3, 5 and 7) on Dams time spent out of the nest with ($F(1,32)=6.4$, $p<0.005$) and this shows that all Dams spent less time out of nest as the litters get older. An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.042$, $p<ns$).

An ANOVA examining the postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=2.8$, $p<0.05$), which shows that stressed Dams treated with imipramine spent more time out of the nest in comparison with vehicle-treated Dams. However, stressed Dams administered imipramine exhibited less time out of the nest on postnatal days 5 and 7. An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.33$, $p<ns$).

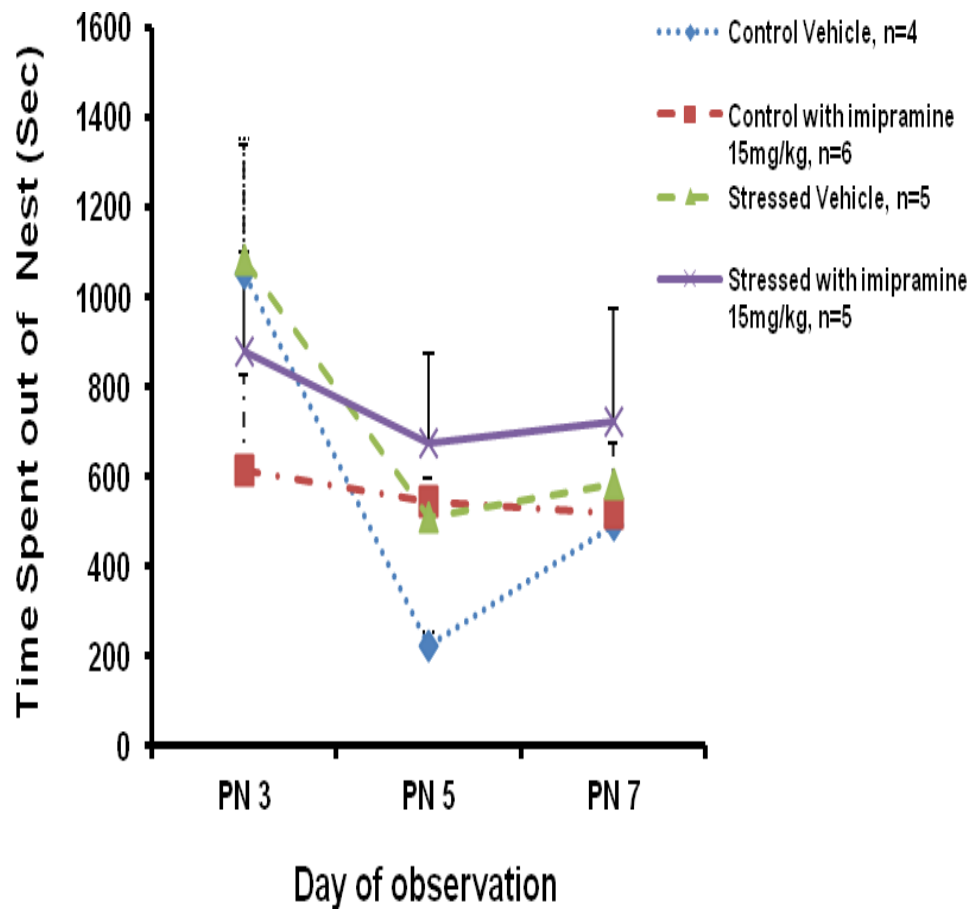


Figure 3-25: Time spent out of Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

3.6.7 TIME SPENT IN NEST BUILDING

Figure 3-26 shows the amount of time the Dams spent nest building. An ANOVA on the data revealed a significant effect of prenatal condition with ($F(1,32)=5.1$, $p<0.04$), showing that stressed Dams spent more time overall nest building compared to control Dams. ANOVA revealed no significant effect of postnatal drug treatment on Dams time spent nest building with ($F(1,32)=0.47$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.49$, $p<ns$). ANOVA revealed no significant effect of test day (3, 5 and 7) on Dams time spent nest building with ($F(1,32)=0.150$, $p<ns$). An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.36$, $p<ns$). An ANOVA examining the postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=6.26$, $p<0.02$) and shows stressed Dams spent more time for nest building in comparison to control Dams. However, stressed Dams treated with imipramine exhibited less time nest building on postnatal days 5 and 7. An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=7.81$, $p<0.01$) and shows that prenatally stressed Dams and treated with imipramine spent less time nest building as the litters get older and this was similar to stressed Dams given vehicle.

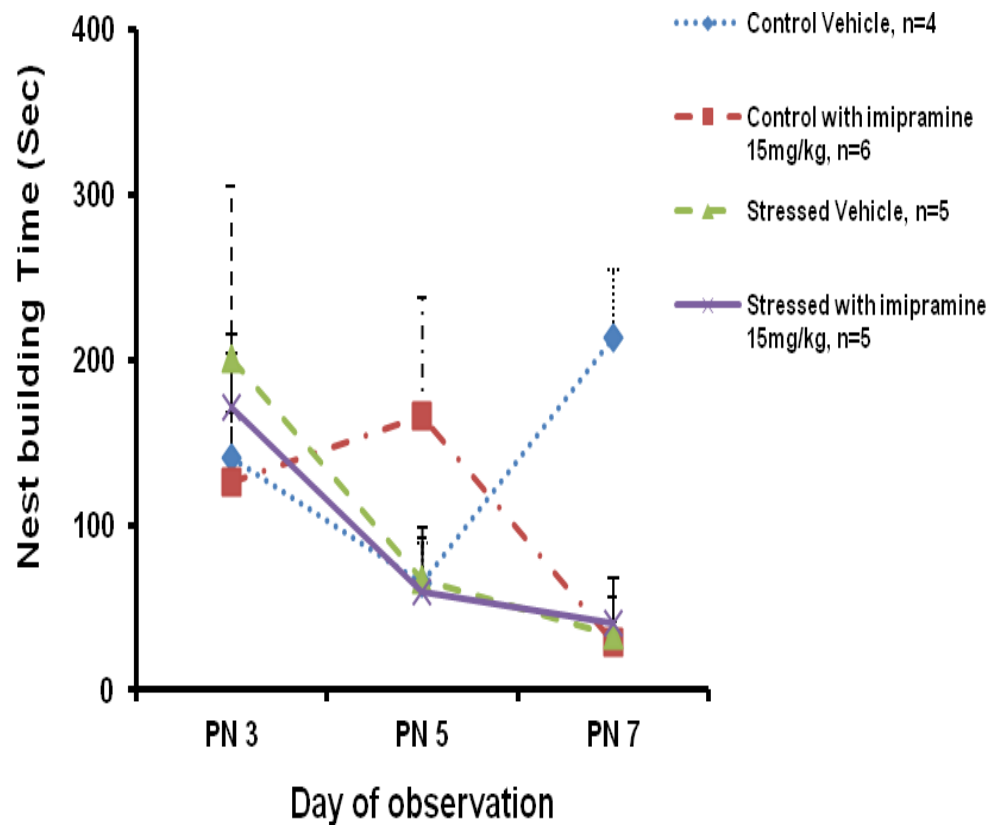


Figure 3-26: Nest Building times for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

3.6.8 DAMS SELF GROOMING INSIDE THE NEST

Figure 3-27 shows the amount of time the Dams spent grooming themselves in the nest. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=0.09$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on time spent self-grooming in the nest with ($F(1,32)=0.93$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=0.34$, $p<ns$). ANOVA revealed a significant effect on Dams times spent self grooming inside the nest over postnatal days (3, 5 and 7) with ($F(1,32)=17.76$, $p<0.0001$) and this shows that Dams increased their self-grooming in the nest as the litters get older. An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.38$, $p<ns$). An ANOVA examining the postnatal drug treatment X day of testing interaction revealed a significant effect with ($F(1,32)=4.74$, $p<0.016$) and this shows stressed Dams treated with imipramine spent more time self grooming in nest in comparison with control administered vehicle. However, stressed Dams administered imipramine exhibited more time self grooming on postnatal days 5 and 7. An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=1.34$, $p<ns$).

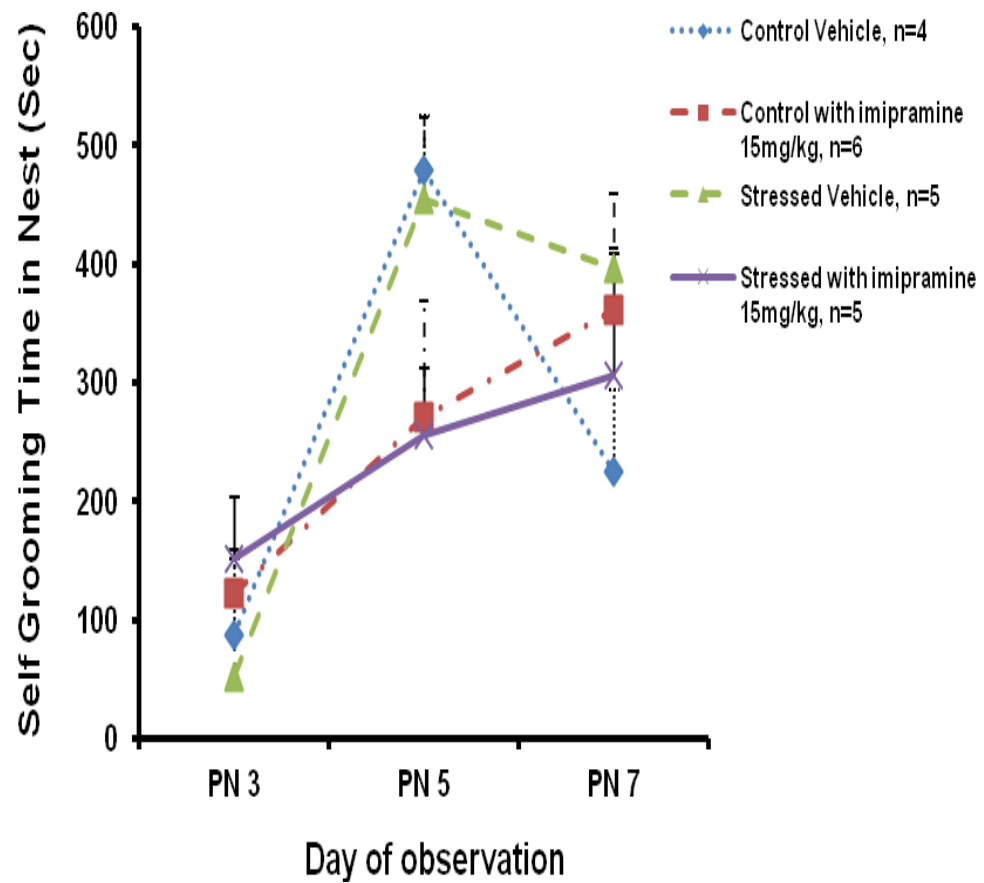


Figure 3-27: Self Grooming times in Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth.
Data shown are Means \pm SEM.

3.6.9 DAMS SELF GROOMING OUT OF THE NEST

Figure 3-28 shows the amount of time the Dams spent grooming themselves outside of the nest. An ANOVA on the data revealed no significant effect of prenatal condition with ($F(1,32)=2.29$, $p<ns$). ANOVA revealed no significant effect of postnatal drug treatment on self grooming times outside of the nest with ($F(1,32)=0.023$, $p<ns$). An ANOVA examining the postnatal drug treatment X prenatal condition interaction revealed no significant effect with ($F(1,32)=2.5$, $p<ns$). ANOVA revealed no significant effect of test days (3, 5 and 7) on Dams times spent self grooming outside the nest with ($F(1,32)=1.19$, $p<ns$). An ANOVA examining the prenatal condition X day of testing interaction revealed no significant effect with ($F(1,32)=0.09$, $p<ns$). An ANOVA examining the postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.152$, $p<ns$). An ANOVA examining the prenatal condition X postnatal drug treatment X day of testing interaction revealed no significant effect with ($F(1,32)=0.66$, $p<ns$).

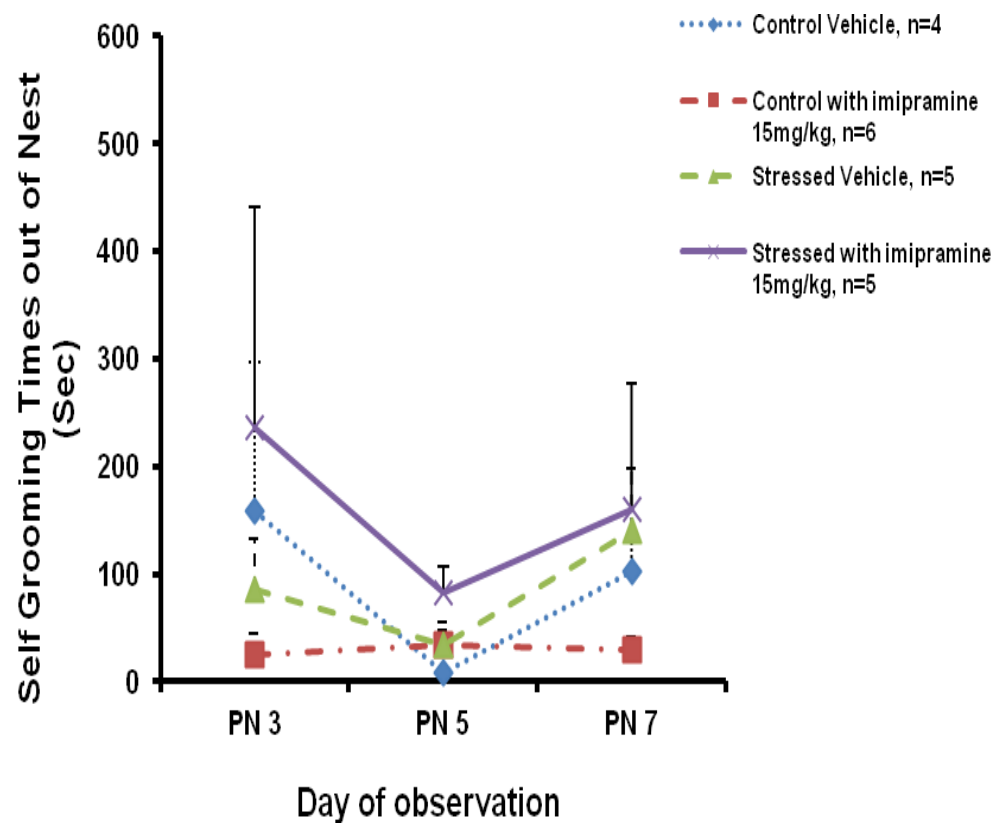


Figure 3-28: Self Grooming times out of Nest for Dams that experienced gestational stress or for controls, and subsequently administered imipramine (15mg/kg) or vehicle, once daily following birth. Data shown are Means \pm SEM.

Schematic Summary of Results Dmas in each behavioural test

1- Dams																
Group	Prenatal Weight	Postnatal Weight (Sertraline)	Postnatal weight (Imipramine)	Prenatal Porsoit (Imipramine)	Postnatal Porsoit (Imipramine)	Elevated Plus Maze			Maternal Behaviour							
						Time to enter	Licking/Grooming	Total time	Arched-back nursing	Supine nursing	Licking/Grooming	Pups retrieval	Time in the nest	Time out of the nest	Nest building	Self grooming inside the nest
Control+Vehicle	✓	X	X	X	X	X	X	X	X	X	✓	X	X	✓	X	X
Control+Drug		✓	✓	X	X	✓	X	X	X	✓	✓	✓	X	✓	X	X
Stressed+Vehicle	X	X	X	✓	X	X	X	X	✓	✓	✓	X	X	✓	X	X
Stressed+Drug		✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	✓	✓	✓	X

188

Schematic Summary of Results Offspring in each behavioural test

2- Offspring							
Group	Postnatal Weight (Sertraline)	Postnatal weight (Imipramine)	Postnatal Porsoit (Imipramine)	Elevated Plus Maze			
				Time to enter	Licking/Grooming	Total time	
Control+Vehicle	X	X	X	X	X	X	
Control+Drug	X	X	✓	X	✓	X	
Stressed+Vehicle	✓	✓	✓	X	X	X	
Stressed+Drug	✓	✓	✓	X	✓	X	

✓- Significant effect
X- No effect

Figure 3-29: Schematic Summary of Results.

CHAPTER FOUR: DISCUSSION

4. DISCUSSION

4.1 EFFECTS ON WEIGHTS

4.1.1 EFFECT OF PRENATAL STRESS ON RAT GESTATIONAL WEIGHTS

Normal weight gain during the course of pregnancy in the rat was profoundly reduced by concurrent gestational stress.

In our study, we found that stressed Dams gained an average weight between 1-3 grams/day (normal weight gain between 5-6 g) which is consistent with other research findings (D'Mello and Liu, 2006; Darnaudéry and Maccari, 2008; Pawluski *et al.*, 2011). Previous work has shown that different stress paradigms e.g. restraint stress (Ward and Weisz, 1984; Alonso *et al.*, 1991a; Vallée *et al.*, 1997), noise and light stress (Freide and Weinstock, 1984), tail and foot shocks (Takahashi *et al.*, 1992a), saline injections (Drago *et al.*, 1999) and immersion in cold water (Velazquez-Moctezuma *et al.*, 1993), can result in significant decreases in gestational weight (Darnaudéry *et al.*, 2004; Van den Hove *et al.*, 2005; van den Hove *et al.*, 2010). In our experiments we have used females weighing between 120-260 grams at the time of conception and it is highly likely that the use of heavier and older females may mask the effect of restraint stress on weight gain during pregnancy in such studies (Pawluski *et al.*, 2011). That is, slow weight gain is much more likely to be noticeable in young, primiparous females, than in older and multiparous females (Ronca *et al.*, 2001; Schroeder *et al.*, 2006).

4.1.2 POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF SERTRALINE

Significant weight differences were also seen postnatally in groups treated with sertraline. All Dams showed modest weight gains over postnatal days 1-10, but the magnitude of this effect was reduced in all animals administered sertraline (Figure 3-2). Previous studies have revealed that the SSRIs can cause weight loss (perhaps) because they have anorectic properties (Moses and Wurtman, 1984; Yen and Fuller, 1992; Clifton and Lee, 1997; Kalia *et al.*, 2000). Hypophagia is one of the main side effects which is known to accompany increased serotonin (5-HT) neurotransmission (whether by administration of 5-HT or its precursors) (Barrett and McSharry, 1975; Rezek and Novin, 1975; Sugrue MF, 1978; Meister, 2007), by 5-HT releasing agents (Pinder *et al.*, 1975; Wurtman and Wurtman, 1977; Meister, 2007), 5-HT receptor agonists (Rezek and Novin, 1975; Aulakh *et al.*, 2012), or by antagonism of 5-HT breakdown (Barrett and McSharry, 1975). Other evidence suggests that serotonergic neurons regulate food consumption in animals (Garattini *et al.*, 1988; Nielsen *et al.*, 1992; Meister, 2007) since 5-HT receptor antagonists (Speight and Avery, 1972; Silverstone and Schuyler, 1975; Weischer ML, 1979) or synthesis inhibitors (Breisch *et al.*, 1976; Meister, 2007) produce hyperphagia.

4.1.3 POSTNATAL DAMS WEIGHTS AND THE EFFECTS OF IMIPRAMINE

Dams receiving imipramine showed negative weight gain over postnatal days 1-10 and only achieved basal weight levels at 10 days postnatally (Figure 3-3), i.e. they initially lost weight regardless of whether they were stressed or controls. A similar finding has been reported previously (Broitman and Donoso, 1978; Hughes and Pither, 1987). This may indicate that the drug has an anorectic effect which diminishes food intake (Broitman and Donoso, 1978; Mogensen *et al.*, 1994). However, it blocks the re-uptake of serotonin and noradrenaline and we might expect hyperphagia in rather than hypophagia (Tucker and File, 1986). Studies suggest that serotonergic axons have the binding sites for imipramine (Palkovits *et al.*, 1981). Chronic treatment with imipramine elevates brain serotonin and its metabolite 5-HIAA in rats (Sugrue, 1983) leading to a disturbance in brain serotonin function and possible dysregulations of appetite, and may be involved in the pathogenesis and aetiology of eating disorders (Kaye, 2008).

4.1.4 OFFSPRING WEIGHT AND THE EFFECTS OF SERTRALINE

The weights of litters from stressed and control Dams were measured to evaluate the effects of restraint stress carried out during the gestation period and any indirect effects of maternal sertraline treatment. Pups weight (based on 10 pups per litter), were measured at various points during the first 10 postnatal days.

Offspring from stressed Dams had a slower weight gain in comparison to the control Dams (Figure 3-4). The low weight gain for stress exposed pups was due to the prenatal stress which may affect Dam's behaviour leading to neglect Dams for the pups and/or the effect of postpartum depression which may be induced by prenatal stress.

However, previous reports show that exposure to SSRI's through breast milk does not affect infants' weight (Hendrick *et al.*, 2003) and thus we believe that our mothers engage in less productive feeding.

Our findings show no significant sertraline effects on stressed and control exposed pups and there was no significant interaction between the drug treatment and prenatal condition. SSRI's are the recommended drug of choice during breastfeeding (di Scalea and Wisner, 2009; Sharma, 2011).

Other studies have found that in humans, females who breast feed, and take fluoxetine, show no changes in platelet 5HT (Epperson *et al.*, 2003). Some studies suggest that antidepressant drugs can pass into breast milk, but only in small amount (Weissman *et al.*, 2004; Davanzo *et al.*, 2011).

However, the drug's milk/plasma (M/P) ratio indicates the estimated concentration of a drug in breast milk. Sertraline, fluoxetine and paroxetine have

M/P ratios of <1.0 which is classed as a low concentration, while fluvoxamine, citalopram and escitalopram exceed this range (Davanzo *et al.*, 2011; Gorman *et al.*, 2012). However, this is not a measure of absolute amount, as this is influenced by several factors including peak milk concentration, dosing schedule, body weight and genetic variability in metabolism which can cause changes in the concentration of an antidepressant in the body (Gentile *et al.*, 2007; Davanzo *et al.*, 2011).

The monoamines have a well-documented ontogeny for whole brain innervation synapse formation and transmitter content. At birth, both NA and DA are clearly present and much of their ascending pathways are present and functioning. i.e. Both ventral and dorsal bundles of the NA neurons of the locus coeruleus and sub coeruleus which ascend to hypothalamus, limbic areas and frontal cortex are present.

In addition, the nigro-striatal, meso-accumbens and meso-cortical DA systems also are well described. However, the 5-HT system is much less existent. Transmitter levels are low in the median and dorsal the raphé nuclei and the ascending pathways are sparse and less pronounced. Indeed the hypothalamic projections of the raphé are only visible following incubation in high-5HT buffers. The rapid growth differentiation of 5-HT projection sites begins at approximately 10-14 days postnatally and culminates in adult levels by about 4 weeks postnatally. This anatomical evidence would suggest that 5-HT drugs in milk would not be expected to have profound effects in neonatal pups, but rather that our results follow maternal behaviour changes.

There are conflicting data, which show that maternal stress affects lactation in both human and animals and when mothers have depressive disorders this can cause the diminished breastfeeding and might decrease milk production (Dewey, 2001; Hatton *et al.*, 2005; Kalueff *et al.*, 2008).

It has been hypothesized that there are two mechanisms explaining the relation between lactogenesis and stress; 1) The release of oxytocin, prolactin and progesterone hormones decrease following; these hormones regulate production and expression (Larsen and Grattan, 2012).

Studies suggested that the oxytocin secretion can be induced via 5-HT receptors in the central of serotonergic neurons (Raap and Van de Kar, 1999).

2) Mothers maternal behaviour could cause inadequate breastfeeding leading to impair the lactogenesis (Dewey, 2001).

Studies suggest that depression may increase the difficulties women experience at start of breastfeeding period (Misri *et al.*, 1997; Henderson *et al.*, 2003; Lau *et al.*, 2007; Dennis and McQueen, 2009).

Psychological bonds between mothers and infants are reinforced by breastfeeding. However, maternal separation may affect this relationship. A study carried out on rats reported that the sucking pattern was affected by maternal separation, due to the decreased interactions between dams and their pups (Brake *et al.*, 1982).

4.1.5 OFFSPRING WEIGHT AND THE EFFECTS OF IMIPRAMINE

Our data show that offspring from stressed Dams treated with imipramine had a slower weight gain in comparison to the offspring of controls Dams. As previous work shows there is a significant retardation of body weight in stress-exposed pups (Broitman and Donoso, 1978).

There was a significant interaction between prenatal condition and drug treatment postnatally; it is obvious that litter weights from Dams administered imipramine showed diminished weight gains over postnatal days 1-10, after which there was a tendency for all litters to be similar in weight (Figure 3-5).

Low birth weights have been reported in others studies associated with prenatal stress (Geber, 1973; Herrenkohl, 1979; Pollard, 1984; Schneider, 1992b; Fameli *et al.*, 1995; Cabrera *et al.*, 1999; Drago *et al.*, 1999; Fonseca *et al.*, 2002; Patin *et al.*, 2002; Lesage *et al.*, 2004; Consoli *et al.*, 2005).

Prenatal stress causes different abnormalities: disturbances in corticosterone levels and locomotor activity (Koehl *et al.*, 1997; Van Reeth *et al.*, 1998; Koehl *et al.*, 1999), dysfunctions in the serotonergic, noradrenergic and dopaminergic systems (Takahashi *et al.*, 1992b; Day *et al.*, 1998) and behavioural impairments (e.g. reduced sexual behaviour and increased anxiety-like behaviour) (Ward, 1983; Rhees *et al.*, 1999). Noradrenaline and serotonin physiology in the CNS are modified by tricyclic drugs caused by an inhibition in their reuptake at synaptic sites (Ross and Renyi, 1975). Given that Dams treated with imipramine show impaired weight gain affect after giving birth, regardless of prenatal condition, three possible explanations might be offered for the observed effects in the pups.

- 1- The mother is experiencing an anorectic effect of the imipramine, fails to consume enough nutrients to maintain or gain weight and likely is unable to produce sufficient quantity or quality of milk for suckling pups.
- 2- The drug may produce subtle alterations in maternal behaviour that appear normal but are actually ineffective. For instance, the Dams nursing postures may appear correct, but she may shift her positions such that the pups fail to attach to the teats sufficiently to feed.
- 3- The drug may pass into the pups via the mother's milk and directly cause an effect on feeding. Neurochemically, imipramine could alter NA or 5-HT (although the paucity of functioning 5-HT synapses in the forebrain suggests this may be tenuous), although we might reasonably anticipate hyperphagia and weight gain instead. If it is a drug-transfer issue then we would suggest a NA-based action if the imipramine to cause the pups to be more sedate and soporific and thus unable to feed sufficiently for the immediate few days following birth. Regardless of the explanations, that proves accurate, by 10 days postnatally the groups differences are absent, reflecting some form of rectification on weight occurs, or tolerance develops to this effect of the drug.

Another study carried by Vallée et al. (1996) suggested that prenatal stress on dams causes disruption in glucose regulation through a lower food intake, leading to an effect on body weight comparing with a control group (Vallée *et al.*, 1996).

Studies on pregnant mice have reported no difference in pups weight who had a reduction in food and water intake for stressed dams (following heat or restraint stress) (Kinsley and Svare, 1986).

In general pup weights from prenatal stressed dams are consistently lower than in non-stressed dams at birth (Morley-Fletcher *et al.*, 2003). However, other studies have reported a decline in body weight after birth following prenatal stress in comparison to control rats (Pollard, 1984; Cabrera *et al.*, 1999).

Our procedure included examining litter sizes, sexing and the cross-fostering pups within their prenatal condition. Thus all control and stressed Dams receive approximately equal numbers of pups, with equality in male and female numbers.

For the present work and in a previous report (Smith *et al.*, 2004) we have not seen an initial weight difference between litters. The difference emerges after birth and resolves by 10 days postnatally. The presence of imipramine is probably not relevant since the same “emergence-resolution” pattern occurs with imipramine (present result) and without imipramine (Smith *et al.*, 2004).

4.2 PRENATAL PORSOLT

4.2.1 PRENATAL PORSOLT TEST IN PREGNANT DAMS

Our findings show that stressed Dams show greater immobility in the Porsolt test on gestational day 17 (Exposure day) (Figure 3-6). Between 10-20 percent of women experience stress and suffer from stress-related disorders during pregnancy, yet our knowledge of how stress impacts the brain and behaviour of the mother during pregnancy is very limited (Pawluski *et al.*, 2011). Our findings indicate that depressive-like behaviours are present before the litters arrive.

Animal models of depression and stress related psychiatric diseases are still undergoing development to create an effective model for use. The nature in research/diagnosis for depression entails emotional and cognitive disruptions and considerable difficulty arises as animals do not exhibit these traits (without inference by researchers).

Most behavioural studies on depression focus on symptoms, such as sleep disturbances, cognitive deficits, lack of motivation or anhedonia (Brummelte, 2008; Weinstock, 2008). These can be modelled in animals and are, but for mood in particular we struggle because we must infer from the rats' behaviours that mood is disrupted.

Researchers use the Porsolt test as a model of depressive-like behaviour (Lucki, 1997; Galea *et al.*, 2001; Cryan *et al.*, 2002; Smith *et al.*, 2004; Cryan *et al.*, 2005; Dalla *et al.*, 2005). This was first developed by Porsolt (Porsolt *et al.*, 1977a; Porsolt *et al.*, 1977b; Porsolt *et al.*, 1978) and measures the active (swimming or struggling) and passive (floating) behaviour of rats or mice in a

container filled with water at a certain temperature (See Methods, page 114). The Porsolt test is a widely used test of antidepressant efficacy (Porsolt *et al.*, 1978) and depressive-like behaviour (Galea *et al.*, 2001; Smith *et al.*, 2004; Dalla *et al.*, 2005; Hinojosa *et al.*, 2006). This test is a very popular method in depression-related research because it is relatively quick, valid and has the possibility for high-through-put testing (Porsolt *et al.*, 1978).

Observations on the time animals spend immobile following treatment with antidepressants show that passive behaviour is significantly reduced. This passive behaviour (immobility) is interpreted as depressive-like behaviour (Kudryavtseva *et al.*, 1991; Lahmame *et al.*, 1997).

The advantage of the Porsolt test in animals for antidepressant treatment/assessment is that observable effects occur within minutes after treatment. Antidepressant effects in humans with the same drugs take weeks for symptoms to abate. The Porsolt is still one of the best models for assessing antidepressants. However, this test has moderate construct and no face validity and its overall application is questionable.

As mentioned earlier “floating” or Immobility in the forced swimming test is considered to be a depressive-like behaviour (Porsolt *et al.*, 1978). It may also represent a strategy to conserve energy in a potentially fatal situation (West, 1990).

Studies have indicated that struggling and swimming behaviours in the rats are regulated by noradrenaline and serotonin, respectively (Detke *et al.*, 1995) and these are explicitly involved in depression and antidepressant action.

In addition we found that immobility times in the stressed Dams were increased both on the exposure day and the experimental day compared to controls (See Figure 3-6); these findings replicate previous research findings, that stressed pregnant females has exhibit greater Immobility during gestation (Frye and Walf, 2004; Szymańska *et al.*, 2009).

Prenatal restraint stress in rats was used in the present study to create model of depression and the procedure for restraint stress is described in the method section (Page 105). The predictive and construct validity of this model was already reported (Koehl *et al.*, 1999; Rao *et al.*, 1999; Lemaire *et al.*, 2000; Morley-Fletcher *et al.*, 2003; Morley-Fletcher *et al.*, 2004). In accordance with previous results, the present study shows that prenatally stressed rats displayed a prolonged immobility time in the Porsolt test prior to birth (Smith *et al.*, 2004; Szymańska *et al.*, 2009) suggesting that maternal depressive-like behaviour begins before litters delivered.

4.3 POSTNATAL PORSOLT (IMIPRAMINE)

Our data have shown that control and stressed Dams had a lower immobility times compared with non-drug treated groups. We have used the Porsolt test because it is widely accepted and the most reliable model for depressive-like behaviour in rodents (Borsini and Meli, 1988; Cryan *et al.*, 2005).

Craft and colleagues (2010) found that postpartum female rats do not spend significantly more time immobile than virgin controls during the first postpartum week. However, they suggested that there are three differences between postnatal dams and virgin controls: postnatal dams defecate more than controls; spend less time swimming; and show less diving in comparison with controls dams (Craft *et al.*, 2010).

Work conducted by Smythe's group (Smith *et al.*, 2004) addressed the role of gestational stress on postpartum depression. In this study they have focussed on gestational stress and/or exposure to high levels of corticosterone during postpartum.

Smith *et al.* (2004) found that gestational stress (restraint stress 1h/daily, days 10–20) induces depressive-like behaviour and changes maternal care in the postpartum dam, thus suggesting that prenatal stress could be used as a model of postpartum depression (Smith *et al.*, 2004).

These results are in line with a later study by O'Mahony *et al.* (2006), who used 1 week of chronic restraint stress (3x/daily) during pregnancy and found higher immobility scores in the Porsolt test after birth (O'Mahony *et al.*, 2006).

Another study by Misdrahi *et al.* (2005) reported that chronic mild stress in mice causes increased corticosterone and estrogen levels in late-term pregnancy

which likely contributes to depressive-like behaviours postpartum (Misdrahi *et al.*, 2005). While gestational or postnatal maternal stress may have elements that potentially serve to model postnatal depression, there are confounding variables for both:

- 1- Gestational stress when the pregnant dam is subjected to chronic application of some aversive stimuli (e.g. restraint, noise, cold, shock), has the benefit of not directly interfering with mother-pup interactions at any point (Weller *et al.*, 1988; F. Champagne, 2000; Weinstock, 2001; Smith *et al.*, 2004; Champagne and Meaney, 2006). Thus, by its nature the source of stress is removed prior to delivery and the dam is not handled during the postnatal period which would entail removing the mothers from their litters (Meaney *et al.*, 1988b; Wakshlak and Marta, 1990; Smythe *et al.*, 1996). This might produce an enforced effect of maternal separation, which by itself may impair/diminish maternal behaviours (Kuhn and Schanberg, 1998; Lehmann *et al.*, 2000; Matthews and Robbins, 2003; Weaver *et al.*, 2007). Loss of heat regulation in the nest might also occur and nest temperatures are known to affect HPA axis programming and maternal behaviour. We have not included maternal separation as a variable in our study because it has complex features that render it difficult to interpret (Kuhn *et al.*, 1990; Huot *et al.*, 2002; Kalinichev *et al.*, 2002; Matthews and Robbins, 2003; Aisa *et al.*, 2007). For instance acute and chronic maternal separation markedly affects the mother-pup interactions and it has greater impact on

fear and anxiety measures, rather than depression (Kalinichev *et al.*, 2002).

- 2- The benefit of using a postnatal stress regime to induce mood alterations in the dams and investigate subsequent mother-pup interactions is that we can be certain that the development, gestational length and birth of the infant are completely natural. That is, we do not have to consider if both the mother and infants have been affected by a gestational stress regime. There are a priori reasons to be circumspect that the gestational stress affect the developing foetus; clearly the smaller litter size and slightly elevated pup mortality, speak concretely to this concern; whereas these are statistically insignificant, they repeatedly appear in our own work and that of others (Guo *et al.*, 1993; Cabrera *et al.*, 1999; Yorty *et al.*, 2004). Obviously there is some direct foetal effect of maternal stress during gestation that impacts on the pups that cannot possibly be attributed to postnatal environment or maternal behaviour. The other repeated effect of gestational stress that is readily apparent but not often included in studies, is the almost immediate (seen within 12-24h of birth) increase in ultrasonic vocalisations (USV) emitted by stress-exposed pups (Bell *et al.*, 1971; Williams *et al.*, 1998a; Morgan *et al.*, 1999; Branchi *et al.*, 2001; Drugan *et al.*, 2009). The presence of USV appears to precede any influence of postnatal maternal behaviours.

Either gestational stress or postnatal stress elicit enduring alterations in the offspring readily measured in the post-weaning days such as affective alterations, HPA axis dysregulation and assorted behavioural changes involving

cognition, nociception and reward recognition (Boyle *et al.*, 2005). For these maternal behaviour is of paramount importance, but we must cognisant of potential influences of maternal stress on either foetal physiology, development, differentiation and vitality as contributing factors to the range of changes seen in stress-exposed offspring as adults (and that interact/affect/contribute to any effects seen in the adult offspring due to maternal behaviour influences).

Our data for the experimental day show that there was a significant effect of postnatal imipramine treatment on immobility times such that prenatally-stressed Dams treated with imipramine exhibited a lower immobility times in comparison with Dams treated with vehicle. This effect means that imipramine restore normal behaviour caused by prenatal stress. This effect has been observed previously prenatally (Alonso *et al.*, 1999; Frye and Wawrzycki, 2003; Morley-Fletcher *et al.*, 2004).

All major classes of antidepressants are sensitive to the Porsolt test, including tricyclic, selective serotonin and noradrenaline reuptake inhibitors, atypical antidepressants and monoamine oxidase inhibitors (Koe *et al.*, 1983; Kulkarni and Mehta, 1985; Borsini and Meli, 1988; Cesana *et al.*, 1993; Nixon *et al.*, 1994; Bourin *et al.*, 1996; Redrobe *et al.*, 1996; Da-Rocha *et al.*, 1997; Sánchez and Meier, 1997).

The immobility in the Porsolt test was considered as a model of depression (Porsolt *et al.* 1977a, 1977b) and thought to represent the psychomotor retardation (PR) shown by many depressed patients. Sobin and Sackeim (1997) have defined PR as a decreasing of motor and mental activity and this give us an important symptom of a major depression (Sobin and Sackeim, 1997).

There has been much advancement in behavioural studies for depression the most notable one being the behavioural immobility assessed by the Porsolt test. It provides adaptive disengagement from the persistent stress of swimming and with active escape forms part of the search-waiting coping strategy (Thierry *et al.* 1984).

Alternatively immobility may reflect a specific state of the mammalian defence repertoire known as “arrested flight” that is related with the psychological construct of “entrapment” in clinical depression (Dixon 1998; Gilbert and Allan 1998). Gilbert and Allan (1998) suggested that the defeat and entrapment scales may be a good set of tools to predict high stress mothers who are in risk for depression (Gilbert and Allan, 1998).

Subjects will actively persist in escape-directed behaviours for longer periods of time if antidepressant treatments are given between the two exposure times. Conversely, in mice, one exposure is sufficient to generate a stable immobility readout that can be countered by acute pre-treatment with antidepressant agents (Borsini and Meli, 1988; Borsini *et al.*, 2002; Cryan *et al.*, 2002).

Another study by Buccafusco and colleagues (2009) reported that the Porsolt test and tail suspension procedures are viewed as simple screening test for antidepressants rather than a model of depression, because the dependent variable (immobility) is a direct reaction to the test itself and does not persist outside the test situation (Buccafusco *et al.*, 2009) i.e. it lacks construct validity. There is no obvious induction of a ‘depressive state’, although there are elements of construct validity (stressful inducing conditions and decreased behavioural output). Prior exposure to the aversive stress which constitutes a

learned helplessness procedure (model of depression) causes long lasting changes in animals, in that they are subsequently less able to learn appropriate escape responses (Telner and Singhal, 1984; Vollmayr and Henn, 2001; Buccafusco *et al.*, 2009).

It has been reported that long-lasting and frequent stress is probably required to produce a lasting change that could be construed as a “depressive state” (Buccafusco *et al.*, 2009).

Previous studies by Borsini *et al.* (1989) and Piras *et al.* (2010) have demonstrated and confirmed the validity of Porsolt test over 2 days. The exposure to a pre-test swim session facilitates the development of immobility, and amplifies the effects of antidepressants during the test session performed 24h later (Borsini *et al.*, 1989; Piras *et al.*, 2010).

Studies report that the Porsolt test results are sensitive to monoamine alterations (Porsolt *et al.* 1977). To summarize, we can consider that “The Porsolt test represents a very specific cluster of stress-induced behaviours (e.g. freezing, aggression, eating) that have no direct, empirical relation to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations (Porsolt *et al.*, 1977b; Holmes and Rodgers, 2003; Petit-Demouliere *et al.*, 2005; Duarte *et al.*, 2006). The Porsolt test is a useful tool to study neurobiological and genetic mechanisms underlying stress and antidepressant responses using a range of mutational techniques in mice such as use of chemical mutagens to induce random mutations and then studying the resulting phenotypes (Porsolt 2000; Lucki *et al.* 2001; Nestler *et al.* 2002).

In a more recent study by McCormick and Green (2012) confirms that the Porsolt is a widely used to model depressive symptomatology in rodents (McCormick and Green, 2012). Others have argued, however, that the test may be better characterized as a measure of passive behavioural reactivity to stress (Holmes, 2003b). Although the face and construct validity of the Porsolt test is questioned (Mosier, 1947; Cronbach and Meehl, 1955; Willner, 1984; Hendrie and Weiss, 1994; Willner, 1997; Townsley and Johnson, 2008), the test has had success as a screen for antidepressants and has predictive validity (Palanza, 2001; O'Neil and Moore, 2003).

4.4 OFFSPRING PORSOLT (IMIPRAMINE)

Our study of offspring from prenatally-stressed or control Dams, administered vehicle or imipramine from postnatal day 1-10 revealed some interesting data for the Porsolt test. Note again that these offspring were tested between 40-50 days of age. We find that offspring of stressed Dams show enhanced immobility in the test and that maternal treatment with imipramine had some effect to reduce this response and that the effect is reduced by treating the mother with imipramine.

If we accept that: 1) enhanced immobility of the stress-exposed offspring reflects a genuine depressive-like state of the animal; and 2) that the imipramine administered to the Dams over postnatal days 1-10, does not pass through the mothers' milk in any significant quantity, then we may reasonably conclude that the drug alters maternal behaviours, tending to normalities and the depressive-like behaviour of the offspring is almost entirely the result of gestationally-induced modifications of maternal behaviour.

Our findings show that there is a significant effect of postnatal maternal drug treatment on offspring immobility times. These differences were not uniquely expressed in the offspring of stressed Dams, as imipramine decreased immobility times in both stressed and control offspring.

Our results suggest that gestational stress produces a depressive-like behavioural effect in the offspring and this behaviour is modifiable with anti-depressant treatment. We suggest that gestational-stress effects observed in the offspring are not directly affected by the *in-utero* stress exposure, but rather

are a result of secondary alterations in maternal behaviour that programme fear, anxiety and depressive-like tendencies of the offspring.

Although the face, predictive and construct validities of the Porsolt model of depression have been reported (Koehl *et al.*, 1999; Rao *et al.*, 1999; Rhees *et al.*, 1999; Lemaire *et al.*, 2000; Morley-Fletcher *et al.*, 2003; Morley-Fletcher *et al.*, 2004) only imipramine and tianeptine (on a few parameters) reverse stress-induced increases in immobility.

Based on current and previous data, we suggest that prenatally stressed rats show depressive-like behaviour in the Porsolt test, i.e. prolonged immobility behaviour. Furthermore, as indicated above imipramine is expected to affect these immobility times, and our present findings with imipramine are in agreement with past research (Szymańska *et al.*, 2009).

In contrast to the significant action of all antidepressants in rats subjected to prenatal stress in the Porsolt test, fluoxetine, mirtazapine and tianeptine had no effect, while imipramine showed only a tendency to decrease immobility in control animals. In line with our results Morley-Fletcher *et al.*, 2003, 2004 showed that imipramine did not change and tianeptine even enhanced immobility times in unstressed rats.

To recapitulate, gestational stress produces offspring that show evidence of depressive-like behaviour via heightened immobility times in the Porsolt test. This depressive-like behaviour responds to anti-depressant drugs as per animal models/screens in which naive rats are subjected to chronic, uncontrollable stress i.e. antidepressant act to reduce evidence of depressive-like behaviour as revealed by diminished immobility times. Thus, gestational stress produces a

“learned helplessness” response identical to that produced in normal rats subjected to a chronic stress regime. Our study now provides important information regarding this phenomenon.

Gestational stress elicits depressive-like behaviours of the offspring via subtle alterations of maternal behaviour and these variant maternal behaviours are also somewhat modifiable by selective antidepressant drugs.

Our data also reveal differences between female and male offspring, such that females from prenatally-stressed Dams’ exhibit lower immobility times compared to males equivalents. Previous including studies show that prenatal stress produces gender-specific effects e.g. sexual behaviour, reproduction and the HPA axis is disrupted more in females than in males (Weinstock *et al.*, 1992; McCormick *et al.*, 1995). Moreover behavioural responses including e.g. exploration, locomotion, anxiety and depressive-like behaviours reported can be gender specific (Braastad, 1998).

Prenatally stressed rats show increased anxiogenic-like behaviour, expressed as a decrease in the time spent in the illuminated areas of an open field (Vallée *et al.*, 1997). The avoidance of anxiogenic environments is in line with data showing decreased exploration in the open arms of the elevated plus-maze (Fride and Weinstock, 1988; Zurita *et al.*, 2000; Maccari and Morley-Fletcher, 2007).

Like the immobility-induced behaviour, studies have also found that the prenatal stress-induced anxiogenic-like behaviour is reduced by major antidepressant drug types (Morley-Fletcher *et al.*, 2003; Morley-Fletcher *et al.*, 2004). Another study (Zienowicz *et al.*, 2006) reported that fluoxetine increases anxiety in some

patients during the first few days of treatment and in experimental animals following a single injection (Belzung *et al.*, 2001; Calatayud and Belzung, 2001; Zienowicz *et al.*, 2006). Fluoxetine administered for 3 weeks had no effect in control rats, but produced anxiolytic behaviour in the offspring of prenatally stressed animals (Zienowicz *et al.*, 2006).

Numerous studies show that different types of stressors applied during the gestational period induces abnormalities in biological and behavioural functions of dams and their offspring (Becker and Kowall, 1977; Barlow *et al.*, 1978; Power and Moore, 1986; Peters, 1988; Maccari *et al.*, 1995b; Weinstock, 1997). Among those dysfunctions are depression, anxiety and changes in HPA axis activity (Alonso *et al.*, 1991c; Weinstock *et al.*, 1992; McCormick *et al.*, 1995; Maccari *et al.*, 2003a; Morley-Fletcher *et al.*, 2003; Smith *et al.*, 2004).

4.5 EFFECTS OF GESTATIONAL STRESS ON POSTNATAL ANXIETY IN DAMS

4.5.1 EFFECTS OF IMIPRAMINE

Elevated plus maze (EPM) behaviour of control and gestationally-stressed Dams were assessed at 4 and 10 days postnatally. Our data showed no differences between stressed and control dams in times to enter the open arms of the EPM. Furthermore, our data show that stressed dams groom longer in the closed arms on day 4, although this effect is not significant by day 10.

According to Leveleki et al. (2006) stress is one of the unavoidable etiological factors of a variety of anxiety disorders (e.g. separation anxiety, generalized anxiety, social phobia, acute stress disorder, Post-Traumatic Stress Disorder (PTSD) (Bassuk *et al.*, 1986; Cox *et al.*, 1991; Rafferty and Shinn, 1991; Pynoos *et al.*, 1999; Lieb *et al.*, 2000; Coupland, 2001; Dierker and Merikangas, 2001; Jetty *et al.*, 2001; Hunt *et al.*, 2002; Kearney *et al.*, 2003; Leveleki *et al.*, 2006).

Acute stressors have also been reported to be effective on the behaviour exhibited by animals on the EPM; for example, immobilization, forced swim, electric shock, surgical stress and saline injection and predator exposure, all enhance anxiety (File, 1996; Hogg, 1996; File, 2001; Mackenzie *et al.*, 2010).

The intent of this assessment was to ascertain whether or not the augmented depressive-like behaviour seen in Dams following gestational stress might be generalized to other fear-associated responses. If such effects were present, would they be sensitive to anti-depressant drugs suggesting that any co-morbidity was primarily depression in origin.

Several studies on anxiety tests suggest that first trial exposure causes an unconditioned fear response, but 2nd trial exposure causes a learning avoidance (Rodgers *et al.*, 1996; File *et al.*, 1998; Pawlak *et al.*, 2012).

The elevated plus-maze (EPM) test is the most widely accepted test procedure to measure anxiety level in animals (Pellow *et al.*, 1985; Rodgers and Dalvi, 1997; Carobrez and Bertoglio, 2005).

The advantage of the test is that it can measure the territory discrimination ability (Patin *et al.*, 2005). The elevated plus-maze is assumed to gauge unconditioned anxiety-like behaviour or, expressed in more specific behavioural terms, unconditioned avoidance behaviour, which has been extensively used as an animal model for anxiety.

It is based on the test-induced conflict between aversion of being exposed to an open and elevated platform and the motivation to explore a novel environment. As a consequence, the less anxious the subject, the more they explore the open arms.

Prenatal stress causes an increase in closed arms exploration in the EPM test in the offspring showing that controls rats are less anxious than prenatally stressed rats (Fride *et al.*, 1986b; Fride and Weinstock, 1988; Weinstock *et al.*, 1988; Wakshlak and Weinstock, 1990; Vallée *et al.*, 1997).

Our findings replicate that to some extent, although to our knowledge the first to assess gestational stress on the behavioural responses of the mothers.

Studies examining the offspring of stressed/control mothers reveal that in rats and primates prenatal stress followed by increased anxiety-like behaviours (Schneider *et al.*, 1992; Clarke and Schneider, 1993; Clarke *et al.*, 1994;

Welberg *et al.*, 2000; Coe *et al.*, 2003). There are sex-dependent changes evident as prenatal stress appears to produce an i.e. anxiogenic response in females and anxiolytic response in males (Luine *et al.*, 2007).

Prenatal Stress appears to masculinize the female performance in anxiety tests and this would be consistent with others who report that prenatal stress induces masculinization of the female offspring in morphology (O'Connor *et al.*, 2003; Sachser and Kaiser, 1996).

Traditional anxiety models do not consistently detect any anxiolytic effect of antidepressant drugs (File and Johnston, 1987; Pellow and File, 1987; Cole and Rodgers, 1995; Rodgers *et al.*, 1997). These models e.g. EPM, conditioned freezing, light/dark transition, fear-potentiated startle, social interaction and conflict test models are widely used to screen the treatment of several anxiety disorders such as PD (J.C. Ballenger J.A. den Boer, 1994), OCD (Piccinelli *et al.*, 1995) and GAD (Rickels *et al.*, 1993; Tyrer and Tyrer, 1994).

For our study we have used the tricyclic drug imipramine. Our findings revealed that there was a significant interaction between prenatal condition and postnatal drug treatment which shows that stressed dams treated with imipramine entered the open arms faster both days 4 and 10. It was the first drug shown to improve anxiety (Klein and Fink, 1962), an observation confirmed by several controlled clinical assays (Liebowitz *et al.*, 1988; J.C. Ballenger J.A. den Boer, 1994).

These studies made clear that only chronic administration of imipramine is effective. According to studies by Pohl *et al.* (1988) and Liebowitz (1989), in the first week of treatment, imipramine may even worsen anxiety (Pohl *et al.*, 1988;

Liebowitz, 1989). In our current study it is clearly evident that our groups of animals given imipramine regardless of prenatal condition strongly support the above mentioned statement.

The total time spent in the closed arms is much higher on the 1st trial compared to the 2nd trial. The most likely explanation for this is that the initial exposure led to habituation of the animals to the closed and open arms.

According to Kahn et al. (1986) chronic, but not acute, imipramine administration has additionally been shown to ameliorate GAD, to an extent comparable to benzodiazepine anxiolytic (Kahn *et al.*, 1986). In contrast, open-arm aversion does not undergo habituation. It is quite obvious that the drug imipramine does have an effect on the open arm exploration.

Accordingly it has been reported that repetitive testing in the elevated plus-maze has been reported to either increase (Treit *et al.*, 1993) or cause no change (Pellow *et al.*, 1985; File *et al.*, 1993) in avoidance performance i.e. changes in time spent in open arms or latencies to enter the open arms.

Literature reviews report that social stress paradigms lead to both increased depressive and anxiogen-like behaviours (Keeney and Hogg, 1999; Berton *et al.*, 2006; Schmidt *et al.*, 2007; Krishnan and Nestler, 2010). However, to get a better understanding of the underlying mechanisms of each disorder separately, animal models producing either a depressed or anxious phenotype are required (Slattery *et al.*, 2012).

When we discuss anxiety as a phenomenon, we need to address other pathological conditions that are related to anxiety. Pathological anxiety consists of heterogeneous classes including, obsessive–compulsive disorder (OCD),

panic disorder (PD), post-traumatic stress disorder, phobias and generalized anxiety disorder (GAD) (American Psychiatric Association, 1994; Teixeira *et al.*, 2000). Researchers have attempted to correlate animal tests with particular types of clinical anxiety for example, OCD (Rapoport *et al.*, 1992), PD (Fontana and Commissaris, 1988; Fontana *et al.*, 1989; Hendrie and Weiss, 1994; Viana *et al.*, 1994; Griebel *et al.*, 1995; Jenck *et al.*, 1995; Molewijk *et al.*, 1995) , post-traumatic stress disorder (Adamec and Shallow, 1993; Rasmusson and Charney, 2006) and phobia (Zangrossi and File, 1992; Zangrossi and File, 1994).

Researchers and the scientific communities have tried to correlate animal models of anxiety and anxiety disorders based on pharmacological responses of animals used clinically to treat human anxiety disorders. The most common anxiolytic used nowadays are the Benzodiazepines (BDZs).

However, the effectiveness's of BDZs in the treatment of PA and GAD is limited now because low-dose SSRIs have been shown to have an anxiolytic profile rather than being antidepressant. The distinction may well come down to acute arousal/worry eliciting anxiety while if this state of arousal persists for some enduring time then depression may emerge.

4.6 OFFSPRING ELEVATED PLUS MAZE AND THE EFFECTS OF SERTRALINE

Our data show that gestational-stress exposed offspring spent less time than control animals in the open arms of the EPM. This effect has been observed previously (Estanislau and Morato, 2005; Fride and Weinstock, 1988). Our findings have also show that offspring of stressed Dams spent less time in the closed arms and less time grooming in closed arms compared to offspring from control Dams.

Females spent less time in the closed arms compared to males. Previous studies have reported that after prenatal stress, females are more anxious than males (Bowman *et al.*, 2004; Gué *et al.*, 2004). Furthermore, Depressive-like behaviour and anxiety can be seen in adult females more than males (Bowman *et al.*, 2004; Richardson *et al.*, 2006).

Patin *et al.* (2005) reported that anxiety-like behaviour increase in male rats after two months of age, whereas, Lordi *et al.* (2000) reports this happens after three months of age. Studies carried out to detect the intrinsic anxiety-like behaviour of rats' suggests that differences in males and females can be seen at 60 days of age in the EPM and open field tests (Masur *et al.*, 1980; Johnston and File, 1991; Imhof *et al.*, 1993; Fernandes *et al.*, 1999).

Our results point towards a trend of the sertraline treated offspring rats to show less anxiety-like behaviour in the elevated plus maze than control rats. This may be because of the actions of serotonin on the amygdala (Davis, 1992).

Anxiety-like behaviour increases during juvenile period after prenatal stress (Vallée *et al.*, 1997). Other studies have reported an increase in anxiety-like

behaviour of prenatally-stressed rats in the elevated plus maze test between postnatal days 60-90 (Patin *et al.*, 2005; Lordi *et al.*, 2000).

Baker *et al.* (2008) found that anxiety-like behaviour could not be detected in stressed/control rats at days 30-31 (Smith and Morrell, 2007; Baker *et al.*, 2008). However, our EPM was carried out between days 36-44.

Gestational stress carried out on rhesus monkeys showed that offspring between days 90-145 exhibit signs of anxiety e.g. irritability, less exploration, clinging to companions and less social interactions (Schneider, 1992a; Clarke *et al.*, 1994).

Studies report that prenatal stress lead to an increased anxiety-related behaviour in primates and rats (Welberg *et al.*, 2000; Coe *et al.*, 2003).

There are only a few behavioural studies measuring anxiety following TCAs and SSRI's treatment. In general, fluoxetine decrease anxiety as revealed by increased open arm exploration seen in treated rats (Konka and Bailey, 2010).

This observation is corroborated with our current study. Meanwhile, that same study showed that control rats spent longer times in the closed arms of the EPM in comparison with fluoxetine treated rats, suggested that they were spending more time in the open arms. But, our study showed that sertraline treated rats spent more time in the closed arms compared controls.

4.7 MATERNAL BEHAVIOUR: EFFECTS OF GESTATIONAL STRESS AND TREATMENT WITH IMIPRAMINE

Maternal behaviour is defined as those behaviours exhibited in preparation for arrival and care of the newborn. Ultimately these behaviours prepare dams for situations requiring protection, nursing and feeding of her infants.

Grota and Ader (1969) defined maternal behaviour of rats as “specific behaviours considered by observer to be maternal in character”. Examples of rat maternal behaviours include: Arched Back Nursing (ABN), supine nursing, licking and grooming of the pups (L/G), pup retrieval, time in the nest, time out of the nest, nest building and Dams’ self grooming.

Maternal behaviour has been extensively studied in the rodent, particularly the rat. From the moment each pup emerges from the birth canal, the mother attends to each with vigorous licking and cleaning, and deposits each under her flank for warmth.

Once all the pups have been delivered, the mother groups them together and adopts a nursing posture, allowing the pups’ equal opportunity to attach to an available teat. By 4-12 hours after birth, the pups will be clean, grouped and have visible ‘milk-bands’ in their abdomens showing that they have fed. Routinely, the dam will select a pup and lick around the genitor-anal region to stimulate urination and defecation. Her presence in the nest maintains the nest temperature at approximately 30-34 °C. She may adopt supine or canopied type nursing positions and often sleeps in a supine position, allowing the pups to feed. The pups have little ability to crawl, but do paddle in an effort to find a teat. Once attached, they maintain a strong grip.

Lactation requires additional energy substrates and this demand necessitates the dams feeding quite frequently. They will walk away from the nest to locate food and water, and a few pups will be dragged out of the nest and detach from the teat. Upon her return to the nest, the dam may re-organise the structure, or move the pups to a new location and build a new nest. In any event she will search for pups (by sight and sound), dig in the bedding and retrieve all of them within approximately 10 minutes. She will leave the nest if she becomes overheated and lay in a remote spot on her back to dissipate heat. Once she has cooled, she may groom herself, feed or return to the nest.

Normal dams give have litters of 8 to 16 pups and the number of males and females are almost equal (Wiesner and Sheard, 1933). Pups stay with their dam until postnatal day 22 and 30, when the pups are self-sufficient at feeding. The dam and her pups normally spend the majority of time in the nest, although this time decreases through postpartum period (Whishaw and Kolb, 2004).

Our data overall, showed that stressed Dams spent less time in an arched-back nursing position (active posture) and relatively more time in a supine posture. Traditionally this has been viewed as being “less nurturant” because the dam is not expending extra energy or effort for her pups’ benefit.

Combined with findings that stressed mothers frequently shift their nest positions, thereby disrupting the contiguity of pup care, the data suggest strongly that these stressed Dams are less interested in their litters. Rather, they seem to be more defensive and guarded, perhaps shifting the nest positions in some virtual attempt to avoid detection from a predator, or being exposed to foreign male rats. She minimizes her energy expenditure towards

the pups, presumably maintaining greater energy reserves to ensure her own health and possibly to maximize her chances to escape should the need arise.

Rats dams generally start building the nest during pregnancy; the architectural features of the nest vary in shape and quality, and we found some differences between stressed and control Dams for this.

Our study documented distinctive patterns of nest location and construction for the various groups; control with vehicle, control with drug, stressed with vehicle and stressed with drug. In general we were able to distinguish among different types of nests and match these with the corresponding group by the quality of the structure. Although our sample size provides limited data they suggest that nesting behaviour largely reflects the Dam's behaviour. For example, non stressed Dams nests showed competent nest building and the pups were neatly encased by a deep nest wall. All the pups were observed in the centre of the nest with a clear, even distribution of nesting material around them. Meanwhile, stressed Dams' nests appeared to have incomplete walls and the nests themselves were loose and dispersed over a larger portion of the cage. The pups were not packed together compared to non stressed mothers' nests (see appendix).

Our data show that all Dams spent less time in a supine position, less licking/grooming and less presence in the nest as the litters got older. Licking and grooming can be observed (e.g. during nursing, before and between retrieval) (Whishaw and Kolb, 2004). However, during the active phase (light off) rat dams spend up to 50% of their time L/G either themselves or their pups (Bolles, 1960). Our results are at odds in other studies where there were no

differences between stressed dams and non stressed in time spent ano-genital licking offspring (Melniczek and Ward, 1994).

Our data show there was an interaction between the postnatal drug treatment and day of testing (3, 5 and 7), suggesting that stressed Dams treated with imipramine spent more time retrieving pups, more time L/G inside the nest, less time in the nest and less nest building especially on PND5 and PND7.

We found for the Porsolt test that prenatally stressed Dams exhibited depressive-like behaviour, which may explain changes in the Dam's maternal behaviour.

Stressed Dams showed less arched back nursing of their pups. Arched-back nursing times were diminished in the Dams treated with imipramine, an effect that can be seen by postnatal days 5 and 7.

Studies report that desipramine is the active metabolite of imipramine; however, desipramine is highly selective for the noradrenaline transporter in the rat (Owens *et al.*, 1997; Cryan *et al.*, 2005). Possibly we do not see a drug response until day 5 because imipramine must be metabolised to desipramine at diminished concentrations, and a response is delayed. Thus the effects of imipramine cannot be seen in first three postnatal days.

Stressed Dams treated with imipramine spent more time in a supine position compared with stressed Dam treated with vehicle; moreover, control Dams treated with imipramine also spent more time in this posture as the pups got older.

We found that stressed Dams exhibited more self-directed behaviours such as licking/grooming themselves in comparison to the non-stressed controls. Our

interpretation for this is either the Dam is more easily distracted or fearful and less inclined to devote energies towards excessive pup care.

Numerous studies have revealed that rat offspring emotive and cognitive behaviours can be affected by prenatal stress (Sontag, 1966; Chapman and Stern, 1979; Stern and Lonstein, 2001; Smith *et al.*, 2004). Studies have found that prenatally stressed dams spend less time caring their pups than themselves (Whishaw and Kolb, 2004).

Francis *et al.* (1999) found that the offspring's behaviour and HPA stress responses were correlated with spontaneous differences in L/G and ABN of the dams. Those offspring from dams which exhibiting more L/G and ABN showed less anxiety, and appeared to produce modest HPA responses to acute stress challenge in comparison to offspring receiving less spontaneous L/G and ABN from their dams (Francis and Meaney, 1999).

Furthermore, changes in the offspring's behaviour could be predicted by the levels of ABN and L/G seen as early as 3 days postnatally.

Retrieval of pups is a measure of the "motivation" of the dams and it is suggested that responsive dams retrieve pups quickly and efficiently, whereas less or non responsive dams retrieve pups much slower (Whishaw and Kolb, 2004).

Kristal (1990) suggested that several systems coordinate maternal behaviour including:

(1)The sensory and cognitive system; (2) motivation and reward system; (3) learning and memory system; (4) emotion and stress system; and (5) motor output system. This means, maternal behaviour can be affected from

neurotransmitters and hormones release during prenatal and postnatal periods that have direct or indirect impact (Kristal, 2009).

Our data lead us to suggest a rationale for why stressed Dams show reduced maternal care of their infant. Gestational stress elicits depressive-like behaviour altering maternal care over postnatal days. It was suggested previously that prenatal stress could be used to model of human postnatal depression (Smith *et al.*, 2004). Our present data corroborate and extend this finding by showing that some stress-induced changes in maternal behaviour are modified by antidepressants.

Beck (1994) reported that certain factors may trigger PPD in women, for example, prenatal stress, child care stress, support and prior life stress (Beck, 1994). Andrews-Fike (1999) suggested that women who have any of those factors should be under observation during the postpartum (Andrews-Fike, 1999). Treatment of PPD is difficult because antidepressants are seen unsafe or unnecessary (Murray and Kelly, 2002). As an illness it still goes unrecognised by health care workers and other family members.

Failure to identify and treat PPD may have serious consequences for mother and child. For instance PPD disturbs mother-infant interactions and leads to slower social and emotional development in children (Field *et al.*, 1990; Stein *et al.*, 1991) and may impair cognitive development and thus educational attainment.

For instance, depressed mothers show greater impatience and dissatisfaction with their infants. During face-to-face interactions, depressed mothers appear to be more insistent that their babies react to play, talking and facial expressions

(Cohn *et al.*, 1990). The babies appear over-whelmed by this assault of noise and visual stimuli and react by withdrawing, crying or turning away from the mothers. Mothers interpret this avoidant response in a negative manner, express exasperation and desist with further contact. They identify the baby's withdrawal as 'I haven't bonded with my child'. The baby learns that to avoid this excessive demand for attention, it needs to avoid eye-contact or become agitated and cry. Thus the baby begins to reinforce the mothers' errant perception of bonding and the aberrant interactions persist.

One of predisposing factors or predictive sign of postpartum depression is anxiety. Women assessed with elevated anxiety during the postnatal period have a greater incidence of postpartum depression (Dalton, 1971; Hayworth *et al.*, 1980; Bridge *et al.*, 1985). However, our data do not show any consistent effects of gestational stress on Dams' anxiety-like behaviour levels. Anxiety-like behaviour during gestation may serve as stressor.

CHAPTER FIVE: GENERAL DISCUSSION

5. General Discussion

In humans, the development of affective disorders (e.g. depression, bipolar disorders, etc) can result from early life adverse experience such as inadequate parental care, abuse, or nutritional deprivation (Bifulco *et al.*, 1997; Harris *et al.*, 1990; Bifulco *et al.*, 2002; Lenze *et al.*, 2008).

The main aim of our investigation was to determine if disruptions of maternal behaviour following prenatal stress exposure might contribute to the behavioural and physiological consequences seen in the offspring. Furthermore, we explored the notion that impaired maternal behaviour in rats might model human postnatal depression, and therefore, whether or not antidepressant drugs would act to restore normal maternal behaviour in prenatally-stressed Dams or otherwise alter them.

If impaired maternal behaviour of the dam represents a form of postpartum depression, then we expected to see higher immobility times in the Porsolt test. If these higher immobility times are valid constructs of rat postnatal depression then immobility times should be reduced by administration of antidepressant drugs. Moreover, if the impaired maternal behaviour is the result of a depressive-like state in the dams, then these behavioural alterations should be ameliorated by antidepressant drugs. Thus, we assessed the effects of prenatal stress, and postnatal antidepressant treatment on dam's maternal behaviour, immobility scores in the Porsolt test, and examined the offspring to ascertain if reinstating or normalising maternal behaviour would prevent gestational stress influences on them.

Fundamentally, it was essential to establish the initial premise that gestational stress impairs maternal behaviour. Rat maternal behaviour is dominated by characteristic features including retrieval and nesting of the pups, arched-back and supine nursing, ano-genital licking and grooming, and in line with Meaney's group regarding spontaneous variations in maternal behaviour, high-nurturant mothers and low-nurturant mothers can be identified. High-nurturant mothers produce offspring which have reduced HPA axis hormone activation in acute stress challenge tests, are less anxious and show better cognitive skills.

The particular maternal behaviours that appear to have paramount importance for programming the offspring with less responsive HPA axis and fear responses are arched-back nursing (ABN) and licking/grooming (L/G).

In our initial report (Smith *et al.*, 2004) we observed that gestational stress exposure during last 10 days of the pregnancy reduced the amount of ABN and L/G exhibited by dams. In the present study, we again see gestational stress effects on maternal behaviour as evidenced by: Stressed Dams spent significantly less time in arched back nursing postures, and more time in a supine position. However, when in the nest, they spent the same amount of time licking and grooming their pups as control Dams did. However, stressed Dams did not spend as much time in the nest.

The lower time spent by the stressed Dam in the nest is indicative itself of a lower level of care. Thus, gestational stress lead to a reduction in the care given by the Dams to their offspring compared to non-stressed control Dams.

We also found that prenatal stress resulted in a slight but non-significant retardation of both the mothers' pregnancy weight gain, and that of the litters.

Such effects were absent after 9-10 days postnatally. The initial diminished postnatal weight gain of the litters again reflects that stressed dams were less attentive to nursing their pups, and devoted less time for it (Barlow *et al.*, 1978; Herrenkohl and Whitney, 1976; Rhees and Fleming, 1981; Williams *et al.*, 1998b; Smith *et al.*, 2004).

There were no effects of prenatal stress on gestation duration, litter size nor on pup postnatal mortality in agreement with our previous report (Smith *et al.*, 2004; Baker *et al.*, 2008).

The Dams treated with antidepressant drugs (sertraline or imipramine) from postnatal days 1-10 had a lower weight gain in comparison to control dams that had been treated with vehicle. Treatment with sertraline of dams had no significant effect on litter weight gain or pup survival. However, Dams administered imipramine showed diminished weight gains throughout the treatment period.

In terms of behavioural changes we found that there was a tendency for stressed Dams to show greater immobility in the Porsolt test measured on gestational days 17 and 18.

Previous work shows that chronic restraint and chronic mild stress increases immobility times in the Porsolt test, and our results agree with these (Platt and Stone, 1982; Harro *et al.*, 1999; Häidkind *et al.*, 2003; Darnaudéry *et al.*, 2004; Smith *et al.*, 2004; O'Mahony *et al.*, 2006). However, there was a significant effect of postnatal imipramine treatment on Dams' immobility times such that Dams administered imipramine exhibited reduced immobility times in comparison with the vehicle treated groups. In general, immobility was

decreased by antidepressant drugs, an effect previously established (Porsolt *et al.*, 1977b; Porsolt *et al.*, 1978; Barros and Ferigolo, 1998; Morley-Fletcher *et al.*, 2004; Buccafusco *et al.*, 2009; Gutiérrez-García and Contreras, 2009; Hesham El Refaey, 2011).

Our results also showed that offspring of stressed Dams exhibited variable immobility times in comparison with control offspring and the offspring of stressed Dams treated with imipramine generally exhibited reduced immobility times. Other studies have found that offspring of prenatally-stressed dams show increased immobility times (Alonso *et al.*, 1997; Alonso *et al.*, 2000; Morley-Fletcher *et al.*, 2003). Hence, we suggest that the effect of imipramine on Dams' maternal behaviour may help to reduce abnormalities in offspring behaviour caused by prenatal stress.

A good animal model is unique to a particular illness or pathological state. Since our intent was exploring the relationship between gestational stress and postnatal depression, we examined maternal behaviour in the EPM. If gestational stress merely results in a variety of affective changes then its utility as a model is questionable. Thus our rationale for testing the intrinsic anxiety state of the Dams was to exclude a general effect on the Dams.

Overall, there were no differences between total time spent in the closed and open arms of the EPM. In general, anxious rats avoid the open arms and thus our Dams showed little evidence of anxiety-like behaviour (Wakshlak and Marta, 1990; Poltyrev *et al.*, 1996; Vallée *et al.*, 1997; Prut and Belzung, 2003; Rimondini *et al.*, 2003; Darnaudéry *et al.*, 2004).

The implications of prenatal stress are broad. Retrospective analysis of emotionally disturbed youths and their maternal influences indicates that stress experienced by the mother during pregnancy contributes both physiological and psychological towards the development of childhood psychopathology (Ward, 1991; Huizink *et al.*, 2004). Similarly, retrospective studies have found associations to exist between prenatal stress and depression, psychosis, hyperactivity and alcoholism (Weinstock, 1997; Weinstock, 2001).

Environmental insults during gestation, illness, stress, fear, abuse, or even pre-existing depression places mothers at risk for developing postnatal depression. Depressed mothers disengage from their infants, and report less bonding with them. The longer-term impact on the children is often harsh and places them at greater risk for health problems, social isolation, poorer socio-economic achievement, and numerous psychopathologies. And yet the relationship between the mother and child remains unexplored in terms of hormonal and physiological systems that trigger and sustain appropriate maternal care and mother-child bonding: what has remained elusive is a biological test system that might give us answers to these important questions; the gestational stress model of postnatal depression may afford us this opportunity.

Given that we are proposing rat gestational stress as a putative model for human postnatal depression it is worthwhile to re-visit the concepts and principles that govern model specifications.

An animal model should reflect the behaviour or illness seen in a human condition, and meet the same criteria as much as possible given the animals' unique overt profile and behavioural repertoires. Thus, externally-observable

behaviours or symptoms in humans should mirror those in rats and vice-versa. The underlying physiological dysfunctions should be almost identical, insofar as the affected structures show evolutionary autology.

Finally, we should be able to modify the rat's physiology a similar manner as we do in humans administered treatments for their affliction. The effectiveness of these treatments in humans should be equally effective at altering the behaviour, or physiological functioning of the rat in its performance under model conditions.

These principles are encompassed in the concepts of face, construct and predictive validities respectively. Many researchers focus only on construct validity in which it is expected that the illness and its underlying pathology are genuinely represented in the animal in structures analogous to those of the human. Stress has long been accepted to be a preceding or permissive factor that promotes and underpins major clinical depression. Animal stress models rely on the theoretical and historically empirical work of Martin Seligman originating some 40 years ago. His rationale was that human depression had as its foundation an underlying component of personal ineffectiveness and misguided belief that internal/external conditions were imposed and immutable. As often expressed in clinically-depressed patients, they were 'helpless' to amend their circumstances, because they perceived that the 'locus of control' over their lives resided externally, beyond their influences.

The abundant literature detailing stress effects on animals confirmed that chronic stress procedures performed in various animal species inevitably increased passivity and helplessness. Animals will stop struggling, remain

motionless and show acceptances of their circumstances, even through opportunities to escape or avoid the aversive situation are made available to them. Thus every animal or rat model of depression measures the degree to which animals become passive following exposure to chronic aversive stimuli. The Porsolt task is a simple and reliable, assessment of passivity (i.e. time spent immobile) that meets and corroborates Seligman's ideas.

A key to understanding the relationship between learned helplessness and human depression is also provided through the pioneering efforts of Aaron Beck (1961). His investigations with depressed patients confirmed that depressive people express openly that they are unhappy, feel unable to alter their circumstances, and that external factors govern their lives. Patients with a history of life-stressors (death in the family, frequent switching of schools, moving house, birth of children, separation), are much more likely to suffer a major depressive illness at least once in their lives.

There is widespread agreement that the Porsolt task sufficiently measures passive states in rats, and that these passive states are elicited by preceding stress. Stress also produces alterations in monoamines and endocrine systems in rats that are largely identical to physiological measures recorded from depressed subjects. Thus following stress passivity is seen as a genuine measure of a depressed affective state of the rat. Therefore, the Porsolt test measures the intrinsic mood (depression) of the rat and our use of it here to measure the learned helpless state of our gestational stressed dams confers construct validity to our gestational stress model of postnatal depression.

Arguably the most important feature of an animal model is its utility to identify and test existing treatments or novel treatments to investigate the physiological and biological constituents of a particular disease or illness. If the model has construct validity then correspondingly, the existing therapies used to treat human patients should alter the animal in a predictable fashion indicative of reduced impairment. Thus for the Porsolt test, stress elicits greater immobility, or passivity, and since our construct defines this immobility as 'depression', then known antidepressant drugs should diminish this immobility response. Conceptually this logic appears unassailable, and indeed there are hundreds of studies that confirm antidepressants act to reduce immobility of rats exposed to the Porsolt, or equivalent tasks, albeit with some variation according to the antidepressant class, dose, preceding stimuli used to invoke stress, and the test parameters of the Porsolt itself (water temperature, depth, duration of test). However, it is worth noting that in human depression, antidepressant effectiveness is on the order of 60-65%, i.e. some 35% of depression is largely refractory to conventional treatments. Parsimoniously we must regard the variations in responsiveness to antidepressants as expected, given the variation of human responsiveness. In the present work we have seen that reductions of maternal behaviours caused by stress were somewhat ameliorated by antidepressant treatment (imipramine) and that elevated immobility times recorded in the Porsolt test also showed signs of abatement. Together these findings would confirm or at least provide positive support that the gestational stress model of postnatal depression has some predictive validity.

Neither the construct or predictive validities render the gestational stress model of postnatal depression particularly compelling, nor have we advanced the science in the field in any particularly novel manner. However, the often overlooked feature of face validity may well represent the significant improvement for the field of stress and depression research that our proposed model offers. The concept of face validity entails examining both the circumstances that predispose the animal and human that leads them to acquire pathological condition. If the outward behavioural manifestations of an illness in rats and humans are identical or closely-resemble, one another, we can claim that on appearances the rat is depressed.

A different model may offer insight to this issue, for example, the overt motor impairments and motor-initiation deficiencies seen in Parkinson disease are faithfully replicated by various lesions targeting the substantia nigra in the rat. The lesioned rat serves as a model for the symptomatology of PD, and has construct validity (PD patients have degeneration of the substantia nigra), and predictive validity as both lesioned rat and PD patient respond to drugs that mimic the transmitter action of the missing neurons. But in no way does the lesioned rat have PD, and Parkinson's patients have not suffered some extraneously produced insult of the substantia nigra. The lesioned rat does not have the illness, and its behavioural perturbations have been manufactured using artificial means.

In our opinion the gestational stress model of postnatal depression may represent the first rat model that sufficiently satisfies face validity criteria. We believe the evidence to date from our work, and that of others, collectively

supports the contention that the gestational stress model has construct and predictive validities insofar as human depression can be theorized to involve 'learned helplessness' and that antidepressants can be claimed to ameliorate depressive symptoms for many patients. Women with postnatal depression disengage from their infants, and in many instances report that they feel less attached to their babies. They provide basic and adequate care for their infants, or indeed allow other family members to undertake maternal care in a secondary fashion.

Gestational-stressed rat dams also show less inclination to expend more energy or effort for their pups' nurturance leading to very similar conclusions that both human and rat have divested themselves of excessive maternal care. Based on this argument by visual inspection we would conclude that as the human is suffering from postnatal depression, then too the gestationally-stressed rat dam is exhibiting a rodent equivalent to postnatal depression. Thus from overt and obvious similarities between human and rat, we arrive at the logical position that the rat is depressed and ergo the rat gestational stress model of postnatal depression satisfies the necessary conditions to possess face validity.

The addition of face validity is of enormous value for research purposes. First, the rat can be used to model human postnatal depression without relying on some extreme, artificial stimulus to initiate the disorder. Admittedly restraint-stress during gestation is an artificial manipulation that has been applied in the present work. But stress has many natural causes both in humans and rats, and these natural sources of stress/distress (e.g. cold, nutritional deprivation, exposure to predators) should also produce similar effects on rat maternal

behaviour as does restraint. The point is that we do not have to artificially manipulate the animal in order for it to mimic the human disorder. As such we can investigate the underlying physiological changes that occur in these stressed dams and expect identical or near identical physiological changes in the human.

Second, the existence of face validity lends considerable support and justification for the already-accepted construct validity. If the construct validity is genuine and based on the overt behaviour rather than matching physiological markers, and a *posteriori* explanations such as learned helplessness, then the explanation of the construct is of less importance. Learned helplessness may or may not be the underlying issue for depression, but if we satisfy face validity, then whatever the name used for the construct is less bothersome. Gestationally-stressed rat Dams show postnatal depressive-like behaviour, regardless of whether or not 'depressive-like behaviour' is accounted for by some nominal description termed 'learned helplessness'.

There is still much work to undertake to explore the areas discussed above, and perhaps none as essential as ensuring that natural or similarly natural stimuli actually elicit the maternal behaviour changes seen following restraint stress in rat Dams. Still we feel that the compelling nature of the apparent existence of face validity propels this particular model to the top of pile.

Finally, it is often reported by researchers that face validity offers little to researchers, because it simply adds the confounding difficulty that we anthropomorphise our animals, and give them human features. Because of this we begin to search for those features and become distracted from the task at

hand. We believe that this is a fallacious argument; surely the essential point of creating an animal model is to replicate as precisely as can be possible all aspects of a human disorder. We do not confer upon the animal human characteristics, we recognise in the animal, as its behavioural and physiological repertoire permit, the features that match those seen in human pathological states.

Models which claim face validity are derided for engaging in anthropomorphical mis-direction, perhaps because face validity is almost non-existent for most animal models. It may reflect some dissonance that critics engage with; since they cannot obtain a model with face validity, its value and importance is not only minimized, but its inclusion or addition to a speculative model is denoted pejoratively as merely an anthropomorphical confound.

Finally, on this point, we fail to appreciate or comprehend the criticism of face validity based on this anthropomorphic argument. It is simply contradictory to ignore the obvious fact that both construct and predictive validities are also based on ascribing to the animal, or rat, the expected theory or explanation for a given pathological state in humans. Of course 'learned helpless' explains the aberrant thought patterns of depressed humans, because the rat in that situation has also learned to be helpless. Antidepressants have some efficacy in the treatment of human clinical depression; ergo we expect learned helpless behaviour of the rat to be relieved following antidepressant treatment. We interpret the animal responses in light of how humans respond, and this is simply another form of anthropomorphising the rat and giving it human

characteristics. This criticism cannot be used to disavow the value of face validity without having a similar outcome for construct and predictive validities. Although a conclusive case for the gestational stress model of postnatal depression in the rat will require further proof and empirical study, we believe our present research adds significant evidence in its support. Whether or not the model will bear up under intensive scrutiny remains to be seen, but its potential utility in postnatal depression research could be of enormous value to researchers, clinicians and patients. It is our hope that other research groups begin examining and refining the model to accelerate its validation.

Suggestions for progressing the work and possible ideas for development

It is expedient to be circumspect with these data, or indeed any behavioural data. We acknowledge that our sample sizes are relatively small, and we base the issue of predictive validity using only single doses of either imipramine or sertraline. However, the best science can offers itself for inspection and replication. It is essential that extramural and independent corroboration. One key element in human postnatal depression is the often unrecognised impact the children. This we feel must an integrated be area for investigation in parallel with the research on maternal behaviours and therapies for postnatal depression. In order to ascertain if early-life adversity carries across generations, i.e. is a veritable epigenetic trait or if early intervention strategies with depressed women alters these putative generative occurrences. As such

future efforts should examine the rat gestational-stress model for generational effects, by carrying out intervention strategies (such as antidepressants), and adoption/cross-fostering of stress-exposed offspring to non-stressed controls, and vice-versa. In this way we may begin to explore and prevent the incidence of familial depression associated with postnatal depression.

Conclusion

Our findings confirm that Dams show depressive symptoms following gestational stress, and that administration of antidepressants to the Dams reduced depressive-like behaviour and increased maternal care.

We propose that rat gestational stress is a putative model for human postnatal depression. Prenatal stress effects on maternal behaviour in the rat Dam represent a novel, and innovative model for human postnatal depression. It had been a goal of the present work to study both maternal behaviour and its modification by gestational stress as one approach by some of our team experimental (in relation to our breeding/methodologies), while team having offspring assessments done in parallel studies by a second team. The current research was structured and extended by one team only, because of staff movements and turnover. This is certainly by not to say that the results obtained the one team approach are unduly impacted by this, and indeed the majority of labs working in this field appear to manage work by establishing a single team. But a disappointment in these overall findings is the inevitable loss of the breadth and range of data by not possessing the resources to comprehensively measure everything that is occurring. This is principally a

feature of the limited data collected for the offspring e.g. infant vocalisations, and dedicated study of how pups contribute directly to any perturbations of their mothers behaviours. Hopefully we can identify the mechanism of transmission, and develop strategies to truncate or prevent the regenerative cycle that contributes to the familial expression of postnatal depression.

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APPENDIX

Animals (Scientific Procedures) Act, 1986
Universities' Training Group

No: 29516

This is to certify that **Ali Alrumaih**
of **University of Bradford**

has successfully completed a programme of training
approved by the Universities' Accreditation Scheme

Modules

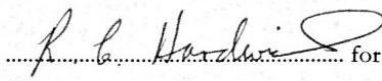
1-3

Species

Mouse

Rat

Guinea Pig

Signed:  for Universities' Training Group
(R C Hardwick - Secretary)

Date: 9 December 2010

Animals (Scientific Procedures) Act, 1986
Universities' Training Group

No: 28371

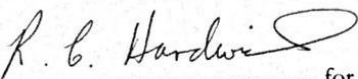
This is to certify that **Ali Alrumaih**
of **University of Bradford**

has successfully completed a programme of training
approved by the Universities' Accreditation Scheme
at the University of Nottingham

Modules

Species

4 _____

Signed:  for Universities' Training Group
(R C Hardwick - Secretary)

Date: 24 May 2010



Home Office

PO Box 31
SHREWSBURY SY3 7WN
Tel 01743 241612 Fax 01743 344691
Web Site <http://www.homeoffice.gov.uk/>

Mr A Alrumaih
School of Pharmacy
Division of Pharmacology
University of Bradford
Bradford
BD7 1DP

Your Reference

Our Reference PHL 40/10095

Date 4 April 2011

Dear Mr Alrumaih

ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

I am pleased to inform you that the Secretary of State has granted your application for a personal licence.

You should check through your licence carefully for any endorsements on this licence in relation to animal types, procedures, establishments, and the conditions attached to it. A personal licence on its own does not authorise you to perform regulated procedures on protected animals. You may only perform the procedures specified on it in the course of a project for which a project licence has been issued under the Act. The use of unauthorised procedures is a breach of the Act and may result in a prosecution and/or revocation of your licence.

You are required to keep a record of all procedures performed under the authority of your licence. This information must be made readily available to the Inspector or Secretary of State when required. If you cease to carry out work requiring a licence (for example leaving the UK to work abroad) you must return your licence to the Home Office.

As soon as you cease to work at the establishment given as the primary availability on your licence, or it ceases to be the place where you wish your licence to be primarily available, you must notify the Home Office, as this change will affect the fees charged. Under the Act you may not delegate the authorities granted to you under this licence to any other person. No other person may perform, either in whole or in part, any procedure authorised by your licence. The only exceptions are certain specific tasks of a non-technical nature. If you have been granted permission to delegate any such tasks, this will be recorded in additional conditions attached to your licence. No other delegation is permitted.

Should you wish any part of this licence to be amended, you must apply to the Home Office giving details of the changes requested and using the Application for change(s) to a Personal Licence form located at: <http://www.homeoffice.gov.uk/science-research/animal-research/>

If a condition of supervision is attached to your licence it is your responsibility to ensure that your supervisor is aware of the authority granted to you under this licence.

Yours sincerely

Mrs C. M. Griffiths



Home Office

No. PIL 40/10095

ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

PERSONAL LICENCE

to

carry out regulated procedures on living animals.

In pursuance of the powers vested in him by the above Act, the
Secretary of State hereby licenses

Mr A Alrumaih
School of Pharmacy
Division of Pharmacology
University of Bradford
Bradford
BD7 1DP

to apply the techniques specified in column a of paragraph 15 of the attached Schedule to the kinds of animals in column b of the same paragraph at the place or places specified in paragraph 14 of this Schedule, subject to the restrictions and provisions contained in the Act, and subject also to the limitations and conditions contained in this licence and to such other conditions as the Secretary of State may from time to time prescribe.

This licence shall be in force until revoked by the Secretary of State and shall be periodically reviewed by him.

Home Office
2 Marsham Street
London SW1P 4DF

For the Secretary
of State

4 April 2011

NB. This licence does not authorise the licensee to perform any of the procedures specified in it unless they are carried out in the course of a project for which there is a project licence in force under the Act.



No. PIL 40/10095
4 April 2011

Personal Licence - Additional Conditions

This licence is subject to the following additional conditions -

The performance of all techniques in the attached schedule shall be given the appropriate level of supervision by the project licence holder or an experienced personal licensee deputed by him/her for such time as may be needed to achieve competence.



Home Office

PO Box 31
SHREWSBURY SY3 7WN
Tel 01743 241612 Fax 01743 344691
Web Site <http://www.homeoffice.gov.uk/>

Dr J W Smythe
School of Pharmacy
University of Bradford
Richmond Road
BRADFORD West Yorkshire
BD7 1DP

Your Reference

Our Reference PPL 40/3415

Date 20 December 2010

Dear Dr Smythe

ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

I am pleased to inform you that the Secretary of State has granted your application for a project licence. The enclosed licence number PPL 40/3415 will be valid until 20 December 2014.

Your attention is drawn to any endorsements on and any additional conditions attached to the licence. As project licence holder you must keep an up-to-date record of every personal licence holder working under the authority of the licence and must be satisfied that their personal licences are valid and are compatible with the procedures and animal types used on the project and with the conditions attached to the project.

Should you wish to vary the terms of this licence in any way, you must apply to the Home Office using the Application for change(s) to a Project Licence form located at:
<http://www.homeoffice.gov.uk/science-research/animal-research/> giving details of the changes requested and quoting the project licence number.

It is the responsibility of the holder of the Certificate of Designation to ensure that all scientific procedures conducted at the establishment are authorised by project and personal licences. You will wish, therefore, to copy the project licence to the holder of the certificate of designation at each establishment where the project is being undertaken and also to each deputy project licence holder.

Yours sincerely

Mrs G M Griffiths

Prenatal DATA sheet

[illegible][illegible][illegible]

Porsolt Testing		
		Day (Gestation)
	DATE	
	Time (minutes)	
	Active	
	Immobile	
	Total Time	
	Comments	

Postnatal Data Sheet													
Cage Reference -													
Animal Reference -													
		Drug		Vehicle		<div>Project Licence Number -40/3415</div> <div>Personal Licence - JWS</div>							
Dosing													
		0	1	2	3	4	5	6	7	8	9	10	11
Date													
Weight													
Pups Weight (g)													
Pups Numbers													
Nest Temperature													
Volume (ml)													
Dosed													
Route of administration													
Porsolt Testing													
		Day (Postnatal)											
DATE													
Active													
Immobile													
Total Time													
Comments													

Table summarizing the breeding success, and distribution of Dams and their litters total numbers of animal were used

*Run#	Dams			Mortality in Dams	Nots	*Pups Number
	Prenatal					
	Mating					
	Total	Pregnant	Non Pregnant			
1	20	17	3	0	3 (embryo resorption)	141
2	20	16	4	1	4 (embryo resorption)	127
3	20	18	2	0	2 (embryo resorption)	110
4	20	18	2	2	Eaten pups (stressed))	86
5	30	25	5	1	Eaten pups (stressed)	167
8	40	37	3	2	During gestation (stressed)	210
Total	150	137	19	6		841

* Two runs (6+7) were terminated due to unspecified infection and prolonged, abnormal pregnancies.

* Cull to 10/mother.

No obvious difference in litter size. Birth numbers always permitted both control + stressed. Litters to be cross-fostered and culled to 10 pups/Dam. Overt differences in live births between the stressed and non-stressed Dams would ha

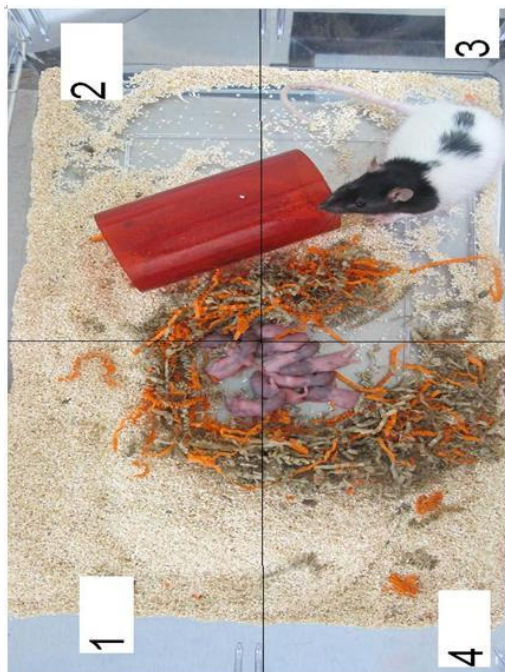


Non Stressed Mother and Offspring.

The nest clearly shows that all the bedding has been used and shows a good example of competent nest building.

The nest is in quadrant 5 which is a central location.

The pups reside neatly encased by a deep nest wall. All the pups are clearly in the centre of the nest with a good clear even distribution of nesting material around them.



Stressed Mother and Offspring

As you can see the nest of the Stressed Mother is ill-defined with no clear walled nest housing the offspring.

The nest itself is loose and dispersed over the majority of the cage.

The pups themselves are not packed together as well as the non stressed mother nest.

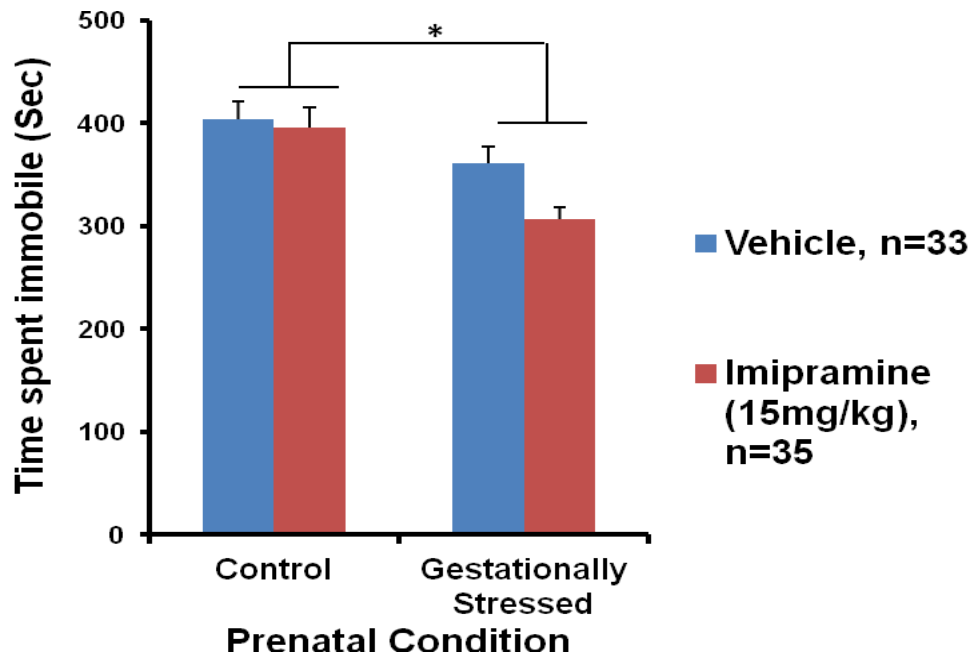
Offspring Porsolt for Females only

Figure below shows the immobility times for offspring (Females) of control and prenatally-stressed Dams. The offspring (Females) selected for this test were chosen randomly from Dams who had undergone restraint stress or were left undisturbed, during gestation and had been treated either with imipramine (15mg/kg) or vehicle (saline) once daily on postnatal Days 1-10. Offspring, females were underwent Porsolt testing between days 40-50 of age.

An ANOVA on the data from Exposure Day (Panel A), revealed a significant effect of prenatal condition on females offspring immobility times with ($F(1,62)=16.7$, $p<0.0001$), which showed that female offspring of prenatally-stressed Dams exhibited lower immobility times in comparison with control. Moreover, there was a significant effect of treatment on females immobility times with ($F(1,62) = 3.8$, $p<0.05$), showed that female offspring of stressed Dams were treated by imipramine exhibited lower immobility times in comparison with those from control Dams. There was no significant effect of females immobility times X prenatal condition interaction for these data with ($F(1,62)=2.04$, $p<ns$). An ANOVA on the data of Experimental Day (Panel B) revealed a significant effect of prenatal condition on females immobility times with ($F(1,62)=10$, $p<0.002$), showing that a female of prenatally-stressed Dams exhibited lower immobility times in comparison with control Dams. Moreover, there was no significant effect of treatment on females immobility times with ($F(1,62)=2.2$, $p<ns$). There was a significant effect of females immobility times X prenatal condition interaction for these data with ($F(1,62)=5.3$, $p<0.02$), showing

imipramine reduced immobility times and effect that was evident only for female of stressed Dams.

A. Exposure Day (10min)



B. Experimental Day (5min)

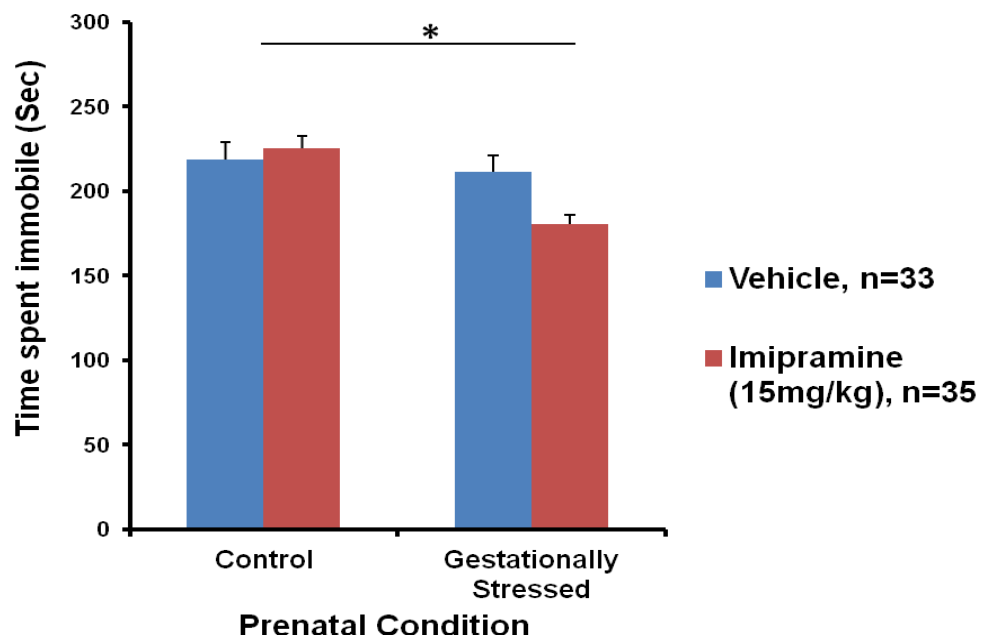


Figure: Immobility times for the Porsolt task recorded from female's offspring of gestationally stressed and Control rat Dams on the Exposure and Experimental Days, when offspring had reached 40-50 days old. Data shown are Means \pm SEM.

* Significantly different, $p < 0.05$, Bonferroni t-test.

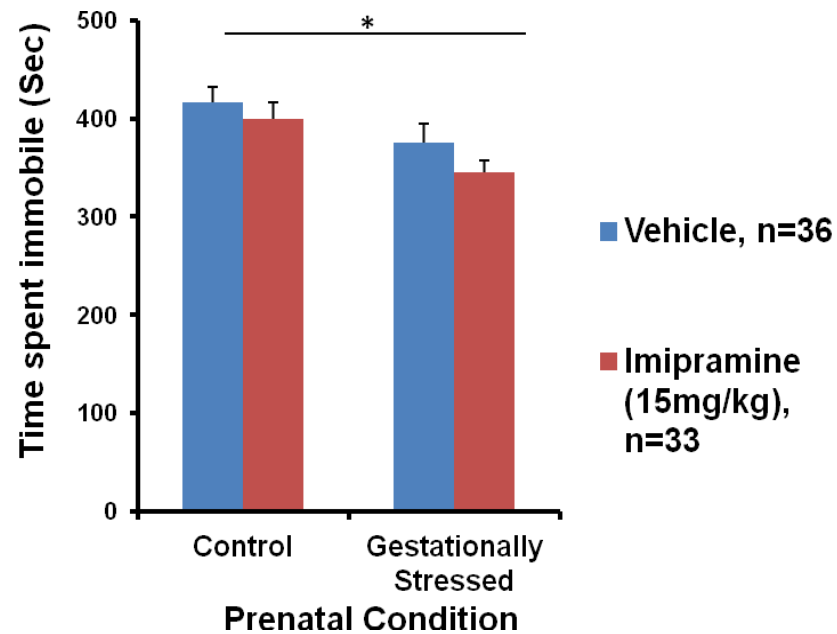
Offspring Porsolt for Males only

Figure below shows the immobility times for offspring (Males) of control and prenatally-stressed Dams. The offspring (Males) selected for this test were chosen randomly from Dams who had undergone restraint stress or were left undisturbed during gestation and the Dams had been treated either with imipramine (15mg/kg) or vehicle (saline) once daily on postnatal Days 1-10. Offspring, males were underwent Porsolt tests between days 40-50.

An ANOVA on the data of Exposure Day (Panel A) revealed a significant effect of prenatal condition on males immobility times with ($F(1,65)=8.7$, $p<0.004$), which showed that male offspring of prenatally-stressed Dams exhibited lower immobility times in comparison with offspring of control Dams. Moreover, there was no significant effect of treatment on males immobility times with ($F(1,65)=2.02$, $p<ns$). There was no significant effect of males immobility times X prenatal condition interaction for these data with ($F(1,65)=0.184$, $p<ns$).

An ANOVA on the data of Experimental Day (Panel B) revealed a significant effect of prenatal condition on males immobility times with ($F(1,65)=4.5$, $p<0.04$), showing that male offspring of prenatally-stressed Dams exhibited lower immobility times in comparison with male offspring of control Dams. Moreover, there was no significant effect of treatment on males immobility times with ($F(1,65)=0.12$, $p<ns$). There was no significant effect of males immobility times X prenatal condition interaction for these data with ($F(1,65)=1.65$, $p<ns$).

A. Exposure Day (10min)



B. Experimental Day (5min)

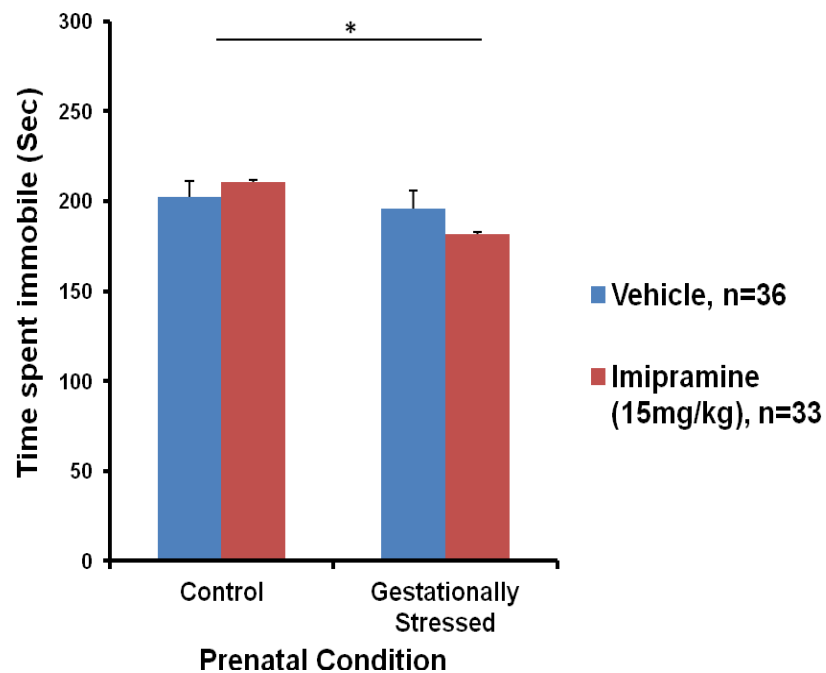


Figure: Immobility times for the Porsolt task recorded from female's offspring of gestationally stressed and Control rat Dams on the Exposure and Experimental Days.

Data shown are Means \pm SEM.

* Significantly different, $p < 0.05$, Bonferroni t-test.

EPM DAMS:

Numerous studies carried out on anxiety found that the most commonly tests used to assess the symptoms of anxiety- like behaviour in rodents are open field (OFT), elevated plus maze (EPM) and light–dark box (LDB) (Ramos, 2008).

Open field test consists of a large arena (larger than the rodent's cage) with high walls to prevent the rodents escaping. In this test mice or rats try to avoid the central of the arena. Thus, it is normally measure the time spent in the center of field, numbers of defecation and the first minutes of rodent's activity, it is used to assess anxiety- like behaviour and general locomotor activity levels in rodents (Hall, 1934; Archer, 1973; Walsh and Cummins, 1976; Gray, 1979; Suarez and Gallup Jr, 1981; Tachibana, 1982; Prut and Belzung, 2003; Stanford, 2007).

The light-dark box (LDB) or black-white box (BWB) consists of two compartments connected to each other through a small opening. One compartment is smaller, black and non-illuminated and the other is larger, white and brightly lit, the test lasted for 5 minutes. Mice or rats explore the black compartment and try to avoid the white compartment, thus, the measures of exploration white compartment (e.g. entrances, time and locomotion) are used as experimental indices of animals behaviour especially anxiety-like behaviour (Crawley, 1981; Costall *et al.*, 1989; Chaouloff *et al.*, 1997; Bourin and Hascoët, 2003).

Table showing animals number in each experiment

Type of experiment	Type of group			
	CV	CD	SV	SD
Dams prenatal weight	46 as Control		42 as Stressed	
Dams postnatal weight (Sertraline)	10	13	10	11
Dams postnatal weight (Imipramine)	10	13	10	11
Offspring weight (Sertraline)	44	68	43	45
Offspring weight (Imipramine)	35	30	35	35
Prenatal Porsolt	19 as Control		22 as Stressed	
Postnatal Porsolt (Imipramine)	8	10	10	9
Offspring Porsolt (Imipramine)	17	15	16	18
Dams EPM (Imipramine)	2	2	4	2
Offspring EPM (Sertraline)	45	67	43	45
Maternal behaviour	4	6	5	5